POPULATION STUDIES OF *CALEDIA CAPTIVA* (ORTHOPTERA:ACRIDINAE) IN SOUTH EAST QUEENSLAND

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DECLARATION

The research carried out in the course of this investigation and the results presented in this thesis are, except where acknowledged, the original work of the Author

Signed:

Chris Moran

C. Moran, March, 1978.

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DEDICATION

To my parents

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ABSTRACT

The *Caledia captiva* species complex consists of four distinct chromosomal races displaying an unprecedented amount of karyoptic divergence. The level of morphological divergence, on the other hand, is extremely low, although multivariate morphometric analysis can separate some of the taxa in discriminant space. In practice, it is not possible to categorise individuals on the basis of external morphology. Chromosomal assays, coupled with a knowledge of the geographical distributions of the races, are the only reliable means of identification.

Two of these chromosomal races, the "Torresian" and the "Moreton", occur in the south east Queensland region and have been studied in detail. Their karyotypes are completely differentiated by rearrangements of centromeric position and differences in the pattern of C-banding. Detailed geographical sampling of both races has revealed that they are parapatrically distributed, with a very narrow zone of hybridization, which extends for more than 150 kilometres, approximately from Maryborough in the north to the vicinity of Brisbane. The "Torresian" race is essentially chromosomally monomorphic, except for populations in the near vicinity of the contact zone. The "Moreton" race, on the other hand, displays an unparalleled level of chromosomal polymorphism. The extent, nature and geographical distribution of the polymorphism in the "Moreton" race is strong evidence for its derivation by introgression from the "Torresian" race.

The extent of the chromosomal differences between these taxa greatly increases the degree of resolution possible in the analysis of the factors responsible for maintaining the narrow contact zone between them. A detailed transect taken across this zone has revealed that the changeover between the races is completed over one kilometre and that about 70% of the change in racial frequencies occurs in the central 200 metre interval. Since the "Torresian" distribution extends for 2,400 kilometres from southern Papua to southern Queensland, and the "Moreton" distribution is at least 300 kilometres long, the abruptness of this change is remarkable.

Both the investigation of the hybrid zone and laboratory experiments indicate that these races inter-breed freely and produce viable F1 progeny. Furthermore, meiotic analysis of the male F1 hybrids, while showing evidence of some genotypic imbalance, has, in general, demonstrated that their ferility is not seriously depressed. However, hybridization experiments and the analysis of gametic disequilibrium of non-homologous chromosomal morphs in the contact zone populations have provided evidence for severe hybrid breakdown. A deterministic model of asymmetrical hybrid breakdown has been developed which explains the narrowness and stability of the hybrid zone, the pattern of nonrandom associations between chromosomes in the hybrid zone populations and the one way introgression from the "Torresian" to the "Moreton" race.

CHAPTER I

Introduction

Numerous cases of karyotypic divergence between closely related species are on record in the literature dealing with speciation (Stone, 1962; White, 1973a,b; Chiarelli and Capana, 1973). Additionally, it can be argued that the inability to demonstrate such differences may often be a reflection of the limitations of the cytological techniques used, rather than the absence of genuine divergence. For example, man, with 46 chromosomes, was originally believed to differ only by a Robertsonian rearrangement from the chimpanzee and gorilla, both of which possess 48 chromosomes. The application of the G and Q banding techniques, however, has revealed that in addition to the obvious Robertsonian difference, there are a further 8 or 9 structural rearrangements in the karyotypes of the three species, including pericentric inversions, paracentric inversions and variation in the size of achromatic blocks (Egozcue, 1975). The greater resolution of these banding techniques thus demonstrates a greater degree of karyotypic divergence than was originally assumed.

In some cases, it is possible to assign a role, either theoretical or demonstrable, to chromosomal rearrangements *per se* as isolating mechanisms between closely related taxa. On the other hand, some such fixed structural differences cannot play any part as an isolating mechanism, since they will not depress fertility when present in the heterozygous state in hybrids. For example, the paracentric inversion differences, which distinguish many species of *Drosophila* (Stone, 1962), by themselves will not reduce the fertility of hybrids. Two major categories of chromosomal rearrangements must therefore be recognized (Table 1.1). The categories are, first, those rearrangements which co-exist in a polymorphic state and secondly, those which are found

CHAPTER 1

Introduction

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* See Appendix

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TABLE 1.1 Categories of chromosomal rearrangements

Type	Characteristics
1. Balanced polymorphisms	- within populations and species - do not depress fertility of heterozygotes a) arise sympatrically or b) by introduction by migration from a geographical isolate or introgression from a closely related species
2. Fixed differences	- between populations and species - result from i) collapsed balanced polymorphism - do not depress fertility in hybrids - cannot act as an isolating mechanism ii) transient polymorphism - depress fertility in heterozygotes - cannot exist as a balanced polymorphism - pass through a transient, unstable polymorphism to fixation or extinction - unlikely to be observed during the transient, polymorphic stage - can act as an isolating mechanism

An area of contention in contemporary speciation theory concerns the question of whether chromosomal rearrangements can initiate the process of cladogenesis in the absence of geographical isolation. The theory of chromosomally initiated speciation or stasipatric speciation has been proposed by White, Blackith, Blackith and Cheney (1967) and White (1968), mainly to account for the high incidence of parapatric associations between chromosomally differentiated taxa of the Viatica group of Morabine grasshoppers. The most important assumption of this model is that the rearrangement differences themselves, independent of any genic differentiation at least initially, must depress the fertility of the heterozygotes. However, chromosomally determined infertility is a necessary, but not a sufficient condition for speciation to have occurred in this way. It need not be interpreted as evidence for a cladogenetic role for the rearrangement. Rearrangements causing a depression of fertility in heterozygotes can quite feasibly be fixed in allopatry, after which the differentiated populations establish secondary contact.

The fertility of hybrids has often not been tested in cases cited as evidence for stasipatric evolution (King, 1977; Hall, pers comm) usually because of the difficulty of maintaining and breeding the parental types in the laboratory. In these cases, it is assumed that the rearrangements do depress fertility. Even when the fertility of hybrids has been tested (White *et al.*, 1967; Craddock, 1971; Mrongovius, 1975), the level of meiotic anomalies in male hybrids has been found to be very low in at least some cases. For example, P(24XY) and viatica 17 differ by the fusion of one of the autosomes to the X chromosome and a pericentric inversion on the X chromosome. They are parapatrically distributed in South Australia, on the mainland and on Kangaroo Island, and are assumed to have evolved stasipatrically (White *et al.*, 1967). However, meiosis in male hybrids is quite normal apart from a 3% increase in univalency over the controls. Furthermore, there is no detectable reduction in fecundity of the F1 hybrids in backcrosses (Mrongovius, 1975). A similar study of hybrids between races of *Didymuria* has revealed only a slight reduction in hybrid fertility, particularly in field collected hybrids (Craddock, 1971) and the author considers that the chromosomal barriers are only partial and insufficient to maintain substantial isolation between races.

A major limitation in the study of chromosomal variation, both within and between species, has been the lack of chromosome markers, which allow rearrangements to be adequately identified. Consequently the best documented examples of karyotypic divergence between taxa, as well as of balanced chromosomal polymorphism, are found in the Brachyterous Diptera, where polytene analysis allows a detailed investigation of even very small rearrangements. Here a notable exception to the apparent universality of karyoptic divergence between closely related taxa has been found in the homosequential species of *Drosophila*. These are species which, by definition, possess the same standard banding sequences on the polytene chromosomes, although additionally, each species may possess characteristic polymorphic, paracentric inversions. Despite this similarity in the banding pattern of the polytene chromosomes, it has been possible to demonstrate major differences in the amount and distribution of constitutive heterochromatin in the mitotic chromosomes of several of these homosequential species. For example, *Drosophila nannoptera* and species "w" differ by the presence of large heterochromatic blocks on the Y chromosome and two autosomal pairs. A rod and a dot autosome in species "w" are seen as large metacentric elements in *D. nannoptera*. A similar difference exists between the Y chromosomes. In *D. nannoptera*, the Y chromosome is a large submetacentric element, composed mainly of heterochromatin (Ward and Heed, 1970). Similarly in the Hawaiian species, *D. silvarentis* possesses 5 rods and 1 dot, whereas *D. heedi* has six rods in its haploid complement (Kaneshiro, Carson, Clayton and Heed, 1973). Neither of these species pairs can be distinguished by their polytene chromosomes, since in these the heterochromatin is under-replicated. Ironically, the members of each species pair can be clearly distinguished by conventional preparations of mitotic chromosomes from brain ganglia, although such mitotic preparations have are only partial and insufficient to maintain substantial isolation between races.

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* See Introduction to Chapter III pp. 45-46 for further discussion of species concept.

a low resolving power for other sorts of karyotypic change. This is not meant to imply that all pairs of homosequential species will have major differences in heterochromatin. However it does illustrate the danger of accepting apparent karyotypic uniformity, using even the well marked polytene system, as conclusive evidence for the lack of chromosomal divergence between taxa.

Sibling species, which are often first recognized because of fixed chromosomal differences, can be of considerable value in assessing the evolutionary importance of karyotypic change. *This is because they are, by definition, groups of reproductively isolated, but morphologically similar organisms. Thus it is possible to assess the relationship between chromosomal divergence and reproductive isolation, without the extra complication of morphological divergence. Although it is not valid to interpret their morphological similarity as evidence for lack of genetic divergence (Mayr, 1963), sibling species in general show a closer genetic relationship than morphologically distinct species. However the level of genic divergence is considerable and one clearly cannot dismiss it as a factor in maintaining reproductive isolation between taxa. Indeed in the case of *Drosophila pseudoobscura* and *D. persimilis*, the sterility of the hybrids in backcrosses has been demonstrated to be the result of genic differences on all chromosomes (Dobzhansky, 1970). A comparison of the level of genic divergence between sibling species and between morphologically distinct species in the *willistoni* group of *Drosophila* (Ayala, Tracey, Hedgecock and Richmond, 1974) has quantified the levels of divergence between species of these types. Coefficients of genetic distance (Nei, 1972) based on electrophoretic data are 0.031 ± 0.007 for local populations within a species, 0.581 ± 0.039 between sibling species and 1.056 ± 0.068 for morphologically distinct species. Even so, it has been pointed out that the loci used in such studies cannot be expected to have any direct role in reproductive isolation between species (Bush, 1975). However since they are assumed to be a random sample of the total genetic variation of the species, this criticism is not necessarily valid. On the other hand, the observation of effectively no electrophoretic, genic divergence between two Hawaiian species, *Drosophila silvestris* and *D. heteroneura* (Sene and Carson, 1977) does make it clear that caution is needed in interpreting these estimates of divergence. In this case, the species are not only morphologically distinct, but in some areas are sympatric. They are reproductively isolated by behavioural mechanisms.

The discovery of sibling species has often depended on cytological analysis of a morphologically uniform group of organisms, although this is not the only means by which they have been detected. Chromosomally distinguishable sibling species have been described in many groups of insects, particularly the Diptera, where they are known for Drosophilids (Stone, 1962), Simulids (Rothfels, 1956; Bedo, 1977) and Culicids (Davidson, 1964; Coluzzi and Sabatini, 1969). They have also been found in the Crustacea (Halfer-Cervini *et al*., 1968), in molluscs (Staiger, 1954), in reptiles (Hall, M.S.) and in many groups of mammals, particularly the rodents (Mathey, 1973; Capanna, Gropp *et al*., 1976). Conversely, homosequential sibling species have been found in Australian blackflies of the *Simulium ornatipes* species complex (Bedo, 1977).

In the Orthoptera, sibling species have been distinguished on several criteria. For example, those of the *Alutacea* group of *Schistocerca* can be identified on subtle, qualitative differences in the structure of the male genitalia (Hubbel, 1960). It is not known whether these siblings are also chromosomally distinct. Many of the chromosomally distinct taxa in the Australian Morabine grasshoppers have no obvious morphological differences between them (White, 1973a) and on the usual criteria would be considered sibling species.

One of the clearest examples in grasshoppers of a group of sibling species defineable on chromosomal grounds is found in the superspecies, *Caledia captiva* (Orthoptera, Acridinae). Here four chromosomal races ("Moreton", "Torresian", "Daintree" and "South east Australian"), which are almost certainly separate sibling species, have been clearly distinguished using conventional cytological techniques on both mitotic and meiotic material (Shaw, 1976). They have been differentiated even further by the technique of C banding (Shaw, Webb and Wilkinson, 1976). In this one superspecies, there is a lack of obvious morphological differentiation but a multiplicity of chromosomal differences, including variation in centromere position and the presence and distribution of constitutive heterochromatin (Table 1.2). In addition, two of the races, the "Daintree" and the "Torresian", are significantly different both in frequency and distribution of chiasmata (Shaw and Knowles, 1977). The other two races have not been tested in this regard. Previous studies of this superspecies (Shaw, pers. comm.) and studies reported here (Chapters 4 and 5) have revealed an array of isolating mechanisms ranging from hybrid breakdown, through complete hybrid sterility and premating, behavioural isolation (Figure 1.1).

The geographical distributions of the four members of the complex are more or less separate, but contiguous with at least one other race. At its northern limit in Cape York, the geographically restricted "Daintree" race is partially sympatric with the "Torresian" race (see Chapter II). The widely distributed "Torresian" race is parapatric with the "Moreton" race in south east Queensland. There appears to be an area of overlap and intergradation between the "Moreton" race and the "South east Australian" race in northern New South Wales, although this area has not yet been examined in detail. Figure 1.2 shows the distribution of the chromosomal races as they are now known.

The taxonomic history of *Caledia captiva* is complicated. The first specimens were collected in 1770 at an unknown location on the east coast of Australia during Cook's voyage and are now lodged in the

Figure 1.1 Hybridization relationships between the four races of *Caledia captiva*

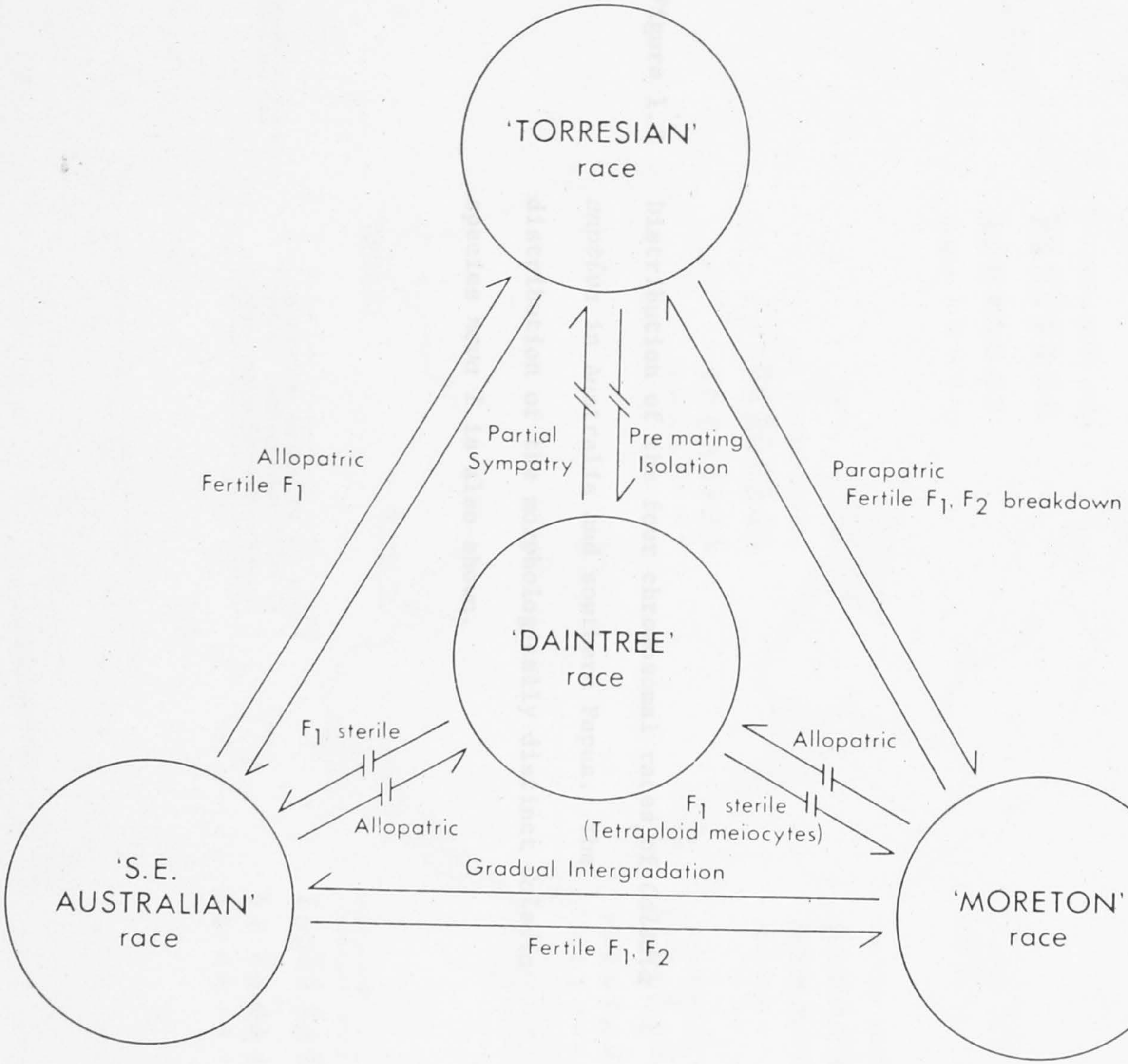
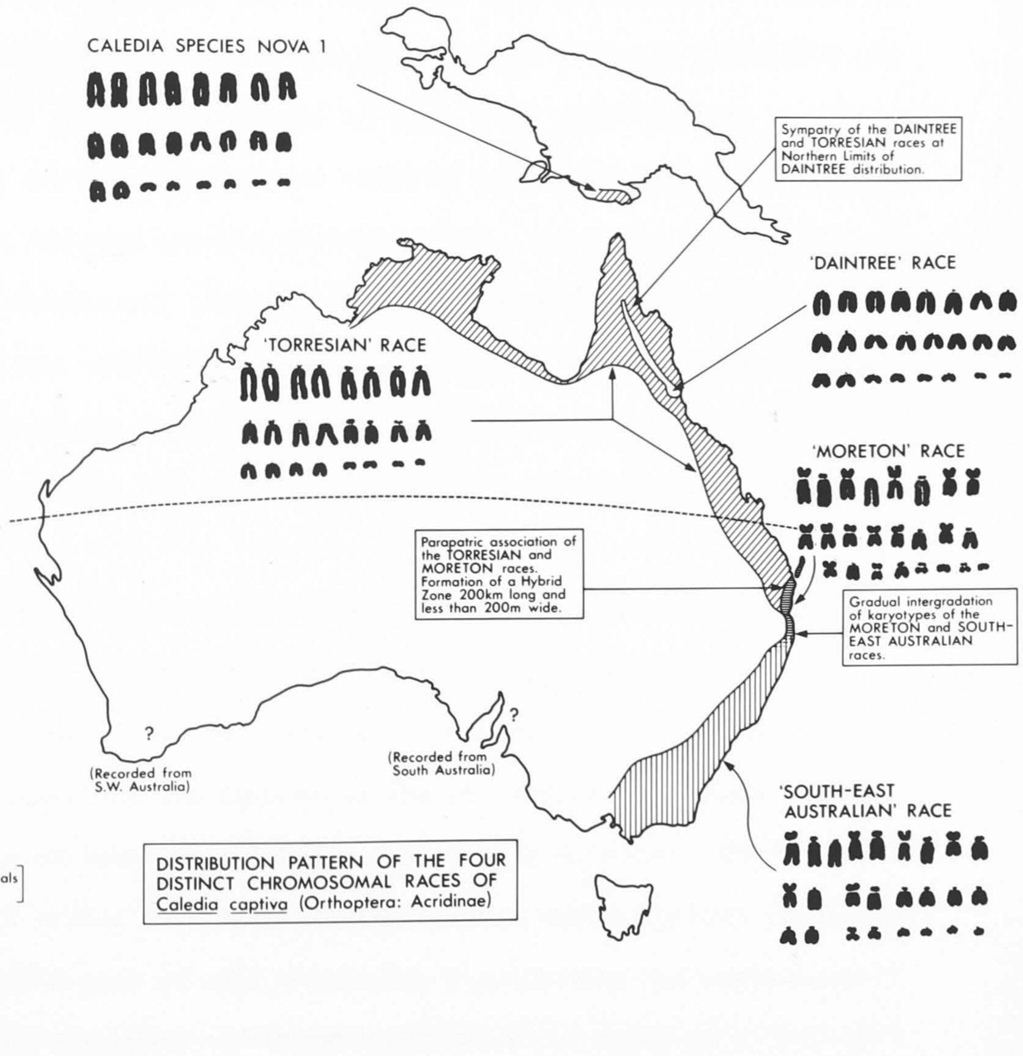

'TORRESIAN' race
'DAINTREE' race
'S.E. AUSTRALIAN' race
'MORETON' race
Allopatric
Fertile F1
Partial
Sympatry
Pre mating
Isolation
Parapatric
Fertile F1, F2 breakdown
F1 sterile
Allopatric
Allopatric
F1 sterile
(Tetraploid meiocytes)
Gradual Intergradation
Fertile F1, F2

Figure 1.2 Distribution of the four chromosomal races of *Caledia captiva* in Australia and southern Papua. The distribution of the morphologically distinct *Caledia* species *nova 1* is also shown.



DISTRIBUTION PATTERN OF THE FOUR DISTINCT CHROMOSOMAL RACES OF *Caledia captiva* (Orthoptera: Acridinae)

[Number of individuals karyotyped 3,700]

Banksian collection of the British Museum. They were named *Gryllus captivus* in 1775 by the Swedish entomologist, Fabricius. For the next 150 years, these original specimens remained in obscurity. Meanwhile Walker (1870) described the male type for the species as *Stenobothrus propinquus*. King Georges Sound was given as the type locality, although *C. captiva* has not since been collected from the southern part of Western Australia. Subsequently Bolivar (1914) described the type for the genus, *Caledia propinqua*, using specimens from Queensland. Finally in 1925, Uvarov examined the two specimens in the Banksian collection and the male type of Walker. He had no doubt that they belonged to the same species and so, according to the rules of taxonomic precedence, arrived at the name, *Caledia captiva* (Uvarov, 1925). However, in the light of the recent chromosomal studies, there is an obvious need for a taxonomic revision of the species complex. A morphologically and chromosomally distinct but as yet undescribed species is also known from southern Papua (Shaw, 1976). *C.* species *nova 1* has not, however, been found in Australia.

It is the purpose of this thesis to describe detailed field and laboratory studies involving the "Torresian" and "Moreton" races of *Caledia captiva*. These two races show a unique pattern of karyotypic divergence involving all members of the chromosome complement and appear to be involved in the early stages of speciation. They are particularly suited for field studies because they have high population densities and a more or less continuous distribution for approximately six months of the year. A capture-mark-recapture study of a "Moreton" population from Mary Smokes Creek revealed a population density of the order of 3,000 adult individuals per hectare (Craft, pers. comm.). Similar densities can be attained by "Torresian" populations although probably for a shorter period of the year.

The studies reported here involve four major components:

1. A detailed description of the cytological characteristics of the "Torresian" and "Moreton" races, including a map of the geographical distribution of the polymorphic chromosomal forms found within them.
2. A mapping of the contact zone between the "Torresian" and "Moreton" races in south east Queensland and a correlation with ecological variables in this region.
3. A description and analysis of inter-racial interactions both in a natural hybrid zone and in laboratory hybrids, from the level of hybrid meiosis to the structure of the contact zone.
4. An analysis of the degree of morphological divergence between the "Moreton" and the "Torresian" races and a comparison with the morphological characteristics of the other two members of the species complex and also of *C.* species *nova 1.*

In the course of these studies, observations have been made on the cytogenetic aspects of speciation, reticulate evolution and genetic organization. In particular, the extent of the chromosomal differences between the "Moreton" and "Torresian" races has led to a detailed consideration of the interrelated problems of hybridization, introgression, and the evolution and significance of isolating mechanisms and hybrid zone structure.

The karyotypic differences between the "Moreton" and "Torresian" races will be described in detail in Chapter 2, but it is worthwhile discussing the nature of the rearrangement differences in the context of chromosomal variation in grasshoppers generally. Acrocentric and telocentric chromosome complements have been found in about 95% of the Acrididae which have been examined (White, 1973a) and are most likely primitive for the family. The presence of submetacentric and metacentric chromosomes, like those found in the "Moreton" race, is not uncommon in species of the Trimerotropine grasshoppers of North America (Carothers, 1917; Wenrich, 1917; King, 1923; Coleman, 1948; White, 1949; White, 1951a,b; Schroeter, 1968; Weissman, 1975; Weissman, 1976). In the Trimerotropines, which belong to the same subfamily as *Caledia*, the rearrangements occur both as balanced polymorphisms and fixed differences between species. Similar rearrangements are found within and between races and species of the Morabine grasshoppers (White, 1973a,b). They have usually been interpreted as derived by pericentric inversion, although it is only in the case of the polymorphic X chromosome in the "Moreton" race that such inversions have been unequivocally demonstrated by C-banding (Shaw *et al.*, 1976). Three break centric transpositions, similar to those which have been demonstrated in *Haplopappus* (Jackson, 1973), provide an alternative explanation for the origin of these rearrangements, but the meiotic consequences in a heterozygote are quite different for the two types of rearrangement.

In several of these cases, the meiotic behaviour of these rearrangements has been observed in heterozygotes. Straight pairing takes place between the standard and rearranged segments at zygotene (Coleman, 1948; White and Morely, 1955). However, because dicentric bridges and large fragments are not produced at anaphase 1 and crossing over does not occur in this region of the chromosome, this is assumed to be non-homologous pairing between mutually inverted segments (White and Morely, 1955; White, 1973b). Inversion loops are not formed and crossing over, leading to duplication and deficiency products, is prevented. There is some evidence from the present study of rare crossing over in the inverted region in inversion heterozygotes, but in general they will suffer little or no reduction in fertility by crossing over in this region. Indeed it is likely that this important characteristic has allowed the evolutionary proliferation of this type of chromosomal rearrangement in these grasshoppers, just as the absence of male recombination and polar body elimination of crossover products in females (Sturtevant and Beadle,

1936) has allowed paracentric inversions to accumulate within and between lineages of *Drosophila*. Presumably the straight pairing mechanism would favour the evolution of paracentric inversions in grasshoppers also though, of course, such inversions cannot be detected by conventional cytological techniques.

It is therefore generally accepted that balanced polymorphisms for pericentric inversions are possible in grasshoppers, because of the straight pairing behaviour which occurs in structural heterozygoes. Such balanced polymorphisms are observed in the "Moreton" and "South east Australian" races of *Caledia*. For this reason alone, it is clear that these rearrangements could not have an initiating role in cladogenesis according to the stasipatric model of speciation. The types of rearrangements required by the stasipatric model are those "which are not known (or only rarely known) to give rise to adaptive polymorphisms in the particular genera or species concerned" (White, 1973b). Since straight pairing in inversion heterozygotes prevents the production of unbalanced gametes by crossing over in the inverted segment, it also precludes the possibility that they could play any direct, divisive role in the speciation process. Pericentric inversions *per se* are incapable of generating a substantial level of hybrid sterility and thus cannot function as isolating agents. The chromosomal rearrangement differences between the "Moreton" and "Torresian" races must therefore have a secondary relationship to the isolation between the races. This is likely to be in terms of altered patterns of co-adaptations resulting from the changed system of genetic organization.

CHAPTER II

Chromosomal Polymorphism, Racial Parapatry and Introgression

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CHROMOSOMA

Population Cytogenetics of the Genus *Caledia* (Orthoptera: Acridinae)

III. Chromosomal Polymorphism, Racial Parapatry and Introgression

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Abstract. The acridine grasshopper, *Caledia captiva* exists as two chromosomal races in south-east Queensland. One of these, the "Moreton" race inhabits the coastal region to the east of the Great Dividing Range. All chromosomes of the complement ($2n = 11II + XO/XX$) have been involved in centromeric rearrangement, which transforms the acro- and telocentric chromosomes into submeta- and metacentric elements. The second, or "Torresian" race is widely distributed through southern Papua, Arnhem Land, Cape York Peninsula and down the east coast of Australia as far south as Brisbane. This race, which is characterised by a completely acro- and telocentric chromosome complement, approaches the "Moreton" race in south-east Queensland where the two races are separated by less than 1 km, along a front of at least 150 km. Evidence is presented to show that chromosome introgression is occurring across the contact zone and this takes place in one direction only, namely the "Torresian" chromosomes are infiltrating into the "Moreton" race but not reciprocally. Furthermore, the introgression of chromosomes across the zone is limited to certain members of the Torresian complement and even then these successful chromosomes show highly variable degrees of penetrance into the "Moreton" race. It is proposed that a "tension zone" exists between these two races which is maintained by the interaction of (a) ecological tolerance differences on either side of the zone and (b) by partial competitive exclusion due to the interracial differences in phenology. This case of parapatric association with limited hybridisation is unique in its clarity due to the marked differences in the appearance of the chromosome complements of these races which permits direct assessment of the behaviour of most members of the genome in hybrids and their derivatives.

Introduction

Parapatric associations between two or more related taxa are generally considered to have established themselves following allopatric divergence (Mayr, 1963).

Such situations have only been reported for a comparatively few organisms and the best known examples involve chromosome differences between populations of relatively sedentary vertebrates (Table 1). For example, two subspecies of *Spermophilus richardsonii* differ by a centric fission, thought to have arisen in isolation. These two subspecies have since made secondary contact along a 25 mile long zone, with evidence of hybridisation at one point along the zone (Nadler et al., 1971). Similar examples have been identified in *Spalax ehrenbergi* (Wahrman et al., 1969), *Thomomys talpoides* (Thaeler, 1968) and in *Perognathus goldmani* (Patton, 1969). Again the Mexican lizard, *Sceloporus grammicus*, has been identified as six distinct chromosomal populations which differ from one another by one or more centric fissions and which form narrow zones of hybridisation in regions of parapatry (Hall and Selander, 1973).

The only parapatric systems so far recorded among the invertebrates are those of the morabine grasshoppers (White, 1974; Mrongovius, 1975), the phasmatid *Didymuria violescens* (Craddock, 1975) and the grasshopper *Podisma pedestris* (Hewitt, 1975). Here too, chromosomal rearrangements are frequently correlated with the distribution pattern of "races" or "species" and they are considered by White and Craddock to have been important factors in speciation. In all these cases the "races" or "species" have been shown to form very narrow hybrid zones in nature.

Zones of contact between two taxa showing chromosomal divergence are potentially useful in assessing the relative roles of pre- and post-mating isolation mechanisms but, unfortunately, their rarity has precluded comprehensive studies.

We have recently identified a situation in an acridine grasshopper (*Caledia captiva*) which shows unparalleled intra- and inter-population chromosome variation both in terms of chromosome morphology (Shaw, 1976) and in the distribution of constitutive heterochromatin (Shaw et al., 1976). This species has been divided into four distinctive chromosomal "races", occupying different geographical regions in eastern Australia and Papua (see Table 2).

One of these, the "Moreton" race, is located in the narrow coastal zone of S.E. Queensland and northern New South Wales to the east of the Great Dividing Range and here all members of the chromosome complement have been involved in centromeric rearrangement. It is the purpose of this paper to describe in detail the level of inter- and intra population chromosomal variation present within this race and to present evidence of an unusually narrow zone of parapatric hybridisation between it and a second, "Torresian", race which is chromosomally monomorphic and has an almost continuous distribution along the Queensland coastal region, Cape York Peninsula and southern Papua.

This case contrasts markedly with that reported for the morabine grasshoppers since, unlike the latter, *Caledia* has not been adversely affected by the impact of agriculture. Indeed, its distribution in south-east Queensland is extremely wide and population sampling can be adequately carried out. Furthermore, from the available evidence of past climatic variation in this area, we can attempt to reconstruct more precisely the racial origins and the approximate age of the present day distribution pattern. The types of chromosomal rearrangements found in this species, its abundance and widespread distribution provide

Table 1. Representative examples of animal species known to show parapatric distributions between closely related taxa which differ in karyotypic organisation. Note that, in all those cases which show evidence of hybridisation, the zone of hybridisation appears to be extremely narrow

Parapatric species or races	Karyotypic difference between contiguous taxa	Association of two taxa	Evidence of hybrid zone
Spermophilus r. richardsonii and *S. r. aureus* (ground Squirrels)	2n = 34 and 36	25 miles long, 6 miles, or less, wide	2n = 34, 35 and 36 within same colony (Nadler et al., 1971)
Thomomys t. ocius with *T. t. pygmaeus* and *T. t. bridgeri*	2n = 48 but races are karyotypically distinct	"within a few feet of each other"	No hybrids ever found (Thaeler, 1968)
T. t. clusius and *T. t. rostralis*		"within a few feet of each other"	No hybrids ever found (Thaeler, 1968)
Spalax ehrenbergi 4 distinctive chromosomal races	2n = 52, 54, 58 and 60	About 15 miles but not accurately defined	2n = 53 (52 × 54) 2n = 59 (58 × 60) (Wahrman et al., 1969)
Perognathus goldmani 6 chromosomal races (α, β, γ, δ, ε, ζ)	2n = 50, 52, 54 and 56 $\alpha = 52$ (FN = 54) $\beta = 56$ (FN = 56) $\gamma = 52$ (FN = 54) $\delta = 50$ (FN = 54) $\varepsilon = 54$ (FN = 54) $\zeta = 53$ (FN = 54)	Race α and race γ; race α and race δ; race β and race α; race β and race δ Racial boundaries are defined by rivers and streams	2n = 51 [$\alpha(50) \times \delta(52)$] Hybrids found sympatrically with δ race (Patton, 1969)
Peromyscus boylii species group	2n = 48; the number of biarmed chromosomes varies between 2 – 10 3 chromosomal races have been recognised	Within 14 miles	No hybrids found between races of *P. b. levipes*. 1 male hybrid between *P. b. rowleyi* and *P. b. spicilegus*. (Schmidly and Schroeter, 1974)
Uroderma bilobatum (Mammalia: Chiroptera)	2n = 38, 2n = 44	Zone of hybridisation over 200 km wide Parental cytotypes show no areas of sympatry	2n = 39–43 representing F_1 and backcross hybrids. (Baker, Bleier and Atchley, 1976)
Macrotus (Mammalia: Chiroptera)	2n = 40, 2n = 46	Both cytotypes taken from a single locality	No indication of introgressive hybridisation was found. (Greenbaum and Baker, 1976)
Sceloporus grammicus (Iguanidae)	2n = 31–46 Races-S: 6 metacentric pairs of macrochromosomes, 10 pairs of microchromosomes in ♀, 19 micros in ♂ due to X_1X_2Y sex chromosome system P_1: Chromosome 1 polymorphic for fission product. F_6: Centric fission for chromosome 6; F5 + 6: monomorphic for fissions of chromosomes 5 and 6. FM: monomorphic for fissions of chromosomes 2, 4, 5, 6 and a microchromosome; polymorphic for fission of chromosomes 1 and 3. F5: monomorphic for a fission of chromosome 5	P and F_6 S and FM S and F_6 P_1 and F_6 Parapatric contacts involving very narrow zones of hybridisation, in one case	Heterozygotes found between P_1 and F_6 representing F_1 and BC1 individuals. Pure F_6 and P_1 samples have been taken within 200 metres of one another. (Dispersal distance estimated to be 400 m). (Hall and Selander, 1973)

Table 1 (continued)

Parapatric species or races	Karyotypic differences between contiguous taxa	Association of two taxa	Evidence of hybrid zone
Podisma pedestris (Orthoptera: Acrididae)	2n = 22 + XX/XO 2n = 20 + neoX/neoY/neoX/neoX	Approximately 500 metres	Mixed populations containing XO and XY males, neoX/neoX and neoX/X females (Hewitt, 1976)
'viatica' group of Morabine grasshoppers	2n = 19 (viatica 19) 2n = 17 (viatica 17) 2n = 16 (P24XY)	*viatica 19* vs *viatica 17*: Parental karyotypes and F_1 heterozygotes in an area approx. 100 m wide.	3 (F_1) chromosomal heterozygotes (1♀, 2♂)
		viatica 19 vs *P24 XY*: "quite narrow zone of overlap"	1 hybrid (F_1) female collected in zone of overlap
		viatica 17 vs *P24XY*: Hybrid zones of less than 100m	17 F_1 females identified together with parental P24 and *viatica 17* types. Hybrid males cannot be distinguished from parental male karyotypes (Mrongovius, 1975)
Didymuria violescens (Phasmatidae)	2n = 26–40. Variation in shape of X chromosome, variation in sex chromosome mechanism (XO, XY primary and secondary). 10 distinctive chromosomal races, all parapatrically distributed with narrow zones of overlap	Only a few natural hybrids collected. Proximity of parental types not given	2n = 28 (30 race × 26 race) 2n = 27 (28 race × 26 race) (Craddock, 1975)

Table 2. Summary of the karyotypic and biogeographic differences known to exist between the four chromosomal "races" of *Caledia captiva*

Chromosomal race	Distribution	Chromosomal characteristics
"Torresian"	Southern Papua, Arnhem Land Cape York Peninsula, East coast of Queensland as far south as Ipswich. Allopatric with S.E. Australian race	Chromosomes 1, 2, X, 4, 5, 7 and 8 acocentric. Chromosomes 6, 9, 10–12 are telocentric
"Daintree"	Area to the north of Cairns spreading inland as far north as McIlwraith Ranges, Cape York. Sympatric with "Torresian" race in Cape York Peninsula. Allopatric with "Moreton" and S.E. Australian races	All chromosomes distinctly telocentric
"Moreton"	Located in south-east Queensland between Maryborough and northern N.S.W. and to the east of the Mary and Brisbane Rivers. Parapatric with "Torresian" race. Overlaps widely with S.E. Australian race in N.E. New South Wales	All chromosomes identified as acro-telo- or submeta/metacentric. In some populations chromosomes 1, 2, X, 4, 5 and 6 are homozygous metacentric. Evidence from karyotypic variation of limited hybridisation with "Torresian" race along a zone of contact approx. 150 km long and less than 1 km wide
S.E. Australian	South eastern Australia as far west as Melbourne and north around Grafton. Found in coastal areas and up to 3,000 ft. in Brindabella Ranges	Chromosomes 1–4, 6, 7 and 10 acrocentric with larger short arms than those of "Torresian" race. Chromosomes 1–5 and 10 polymorphic for large inversions morphologically similar to "Moreton" race

an ideal opportunity to study the nature of the contact zone, whether transient or permanent, and those mechanisms responsible for its maintenance.

Materials and Methods

Ninety-five populations comprising a total of over 1,300 individuals were sampled and karyotyped from south-east Queensland (Fig. 1 and Table 3). All grasshoppers were injected with 0.05% colchicine in insect saline and, after 8 h, the mid-gut caeca were dissected out and fixed in 3:1 absolute ethanol:glacial acetic acid. In the case of males, the testis was removed and fixed prior to colchicine injection. All individuals were given a code number which, in the case of males, allowed easy cross referencing of both mitotic and meiotic chromosomal variation.

All fixed material was stored at 4° C. Squash preparations were stained in lactopropionic orcein and for each individual, one or two flat, entire cells with no overlapping chromosomes were photographed at a magnification of ×2,000, from which a karyogram for each individual was constructed.

Results

The Karyotypes

The karyotypes of both the "Moreton" and "Torresian" races, as well as their patterns of C-banding, have previously been described (Shaw, 1976). However, since accurate chromosome identification is critical for the construction of meaningful chromosome frequency tables, it is worth reviewing and, in some cases, refining the previously published descriptions. The original description of the "Torresian" or "General Purpose" karyotype is retained in full. Despite the fact that there are variations in the clarity of appearance of the short arms, chromosomes 1, 2, X, 4, 5, 7 and 8 are regarded as acrocentric (Fig. 2). On the other hand, chromosomes 6, 9, 10, 11 and 12 are consistently telocentric. In a subsequent description (Shaw et al., 1976), chromosomes 6 and 7, which are similar in overall length, were erroneously interchanged but this has now been corrected in favour of the original description.

In the "Torresian" race, the short arm of the X chromosome is larger than that of chromosome 1 which, in turn, is larger than that of chromosome 2 (Figs. 2a and 4) The main contention here is whether chromosome 2 is structurally the same as the noninverted chromosome 2 found in the "Moreton" race (compare Figs. 2a and b with 3c and d).

Rare submetacentric forms of chromosomes 4, 5 and 6 have been found in the "Torresian" race (see Fig. 2b) which are structurally identical to "Moreton" submetacentrics but they only occur in "Torresian" samples collected close to the contact zone (see Fig. 1).

A karyotypic description of the "Moreton" race is complicated by the extensive structural diversity of the chromosomes within this race. Each member of the complement has been identified in at least two structural morphs, distinguished by the position of the centromere (Fig. 3).

Both chromosomes 1 and 2 have submetacentric and acrocentric forms which, in the case of the submetacentrics, are not easily distinguished. However, since

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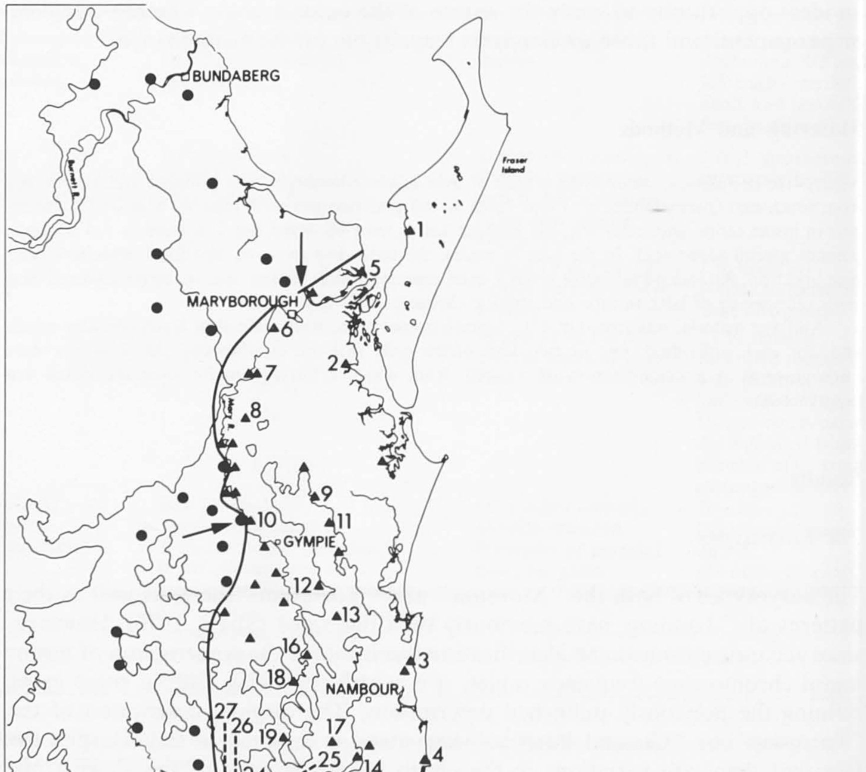

Fig. 1. The distribution of collection sites of the "Moreton" race in south-east Queensland (nos. 1–27). The solid line represents the probable line of contact of the "Moreton" race with the more western "Torresian" race. At four points, these two races are less than 1 km apart. The arrows show the location of "Torresian" populations found to contain a low frequency (1–3%) of "Moreton" chromosomes (either 5, 6, 8, 10 or 11)

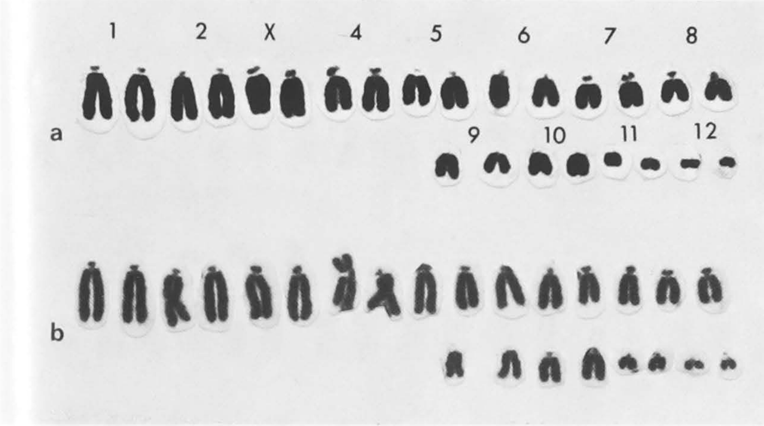

Fig. 2a and b. Karyotypes of the "Torresian" race; (**a**) female mitosis showing the nature and distribution of the acro- and telocentric chromosomes within the karyotype. Note the sequence of the size of the short arms of chromosomes 1, 2 and the X chromosome, (X > 1 > 2). (**b**) Derived hybrid female showing a "Torresian" karyotype with one "Moreton" metacentric chromosome (chromosome 4). This individual was found within a "Moreton" population

the acrocentric chromosomes are quite readily recognised, this does not present any difficulty in classification. Thus, in populations of the "Moreton" race which carry the acrocentric form of the X chromosome, the non-inverted form of chromosome 1 has the largest, and chromosome 2 the smallest, short arm, with that of the X chromosome being intermediate in size, (i.e. 1 > X > 2). This is quite different from the situation in the "Torresian" race where the sequence is X > 1 > 2 (Fig. 4). Telocentric forms of chromosome 2 are infrequently found in some "Moreton" populations but for the purpose of frequency tabulation no distinction has been made between this and the acrocentric form.

The "Moreton" X chromosome, which is the third largest in the complement, is readily identified on arm ratios, both in its metacentric and acrocentric forms (Fig. 4).

Both chromosomes 4 and 5 have acrocentric and submetacentric forms which can be classified by their overall length differences, but not on arm ratios. On the other hand, both inverted and noninverted forms of chromosome 6 are clearly distinguishable from chromosomes 4 and 5 (Fig. 3c and d). The non-inverted form of chromosome 5 is telocentric and the submetacentric form has an arm ratio clearly different from chromosomes 4, 5 and 7 (Figs. 3b and f).

A clear distinction cannot be made between chromosomes 7 and 8 since both their acro- and submetacentric morphs are almost identical in size and morphology (Fig. 3d–f). It is true that the non-inverted acrocentric form of chromosome 8 has a slightly larger short arm that 7 (Fig. 3c) but this does not allow unambiguous assignment when one pair is homozygous submetacentric and the other is structurally heterozygous as in Figure 3d and e. Consequently,

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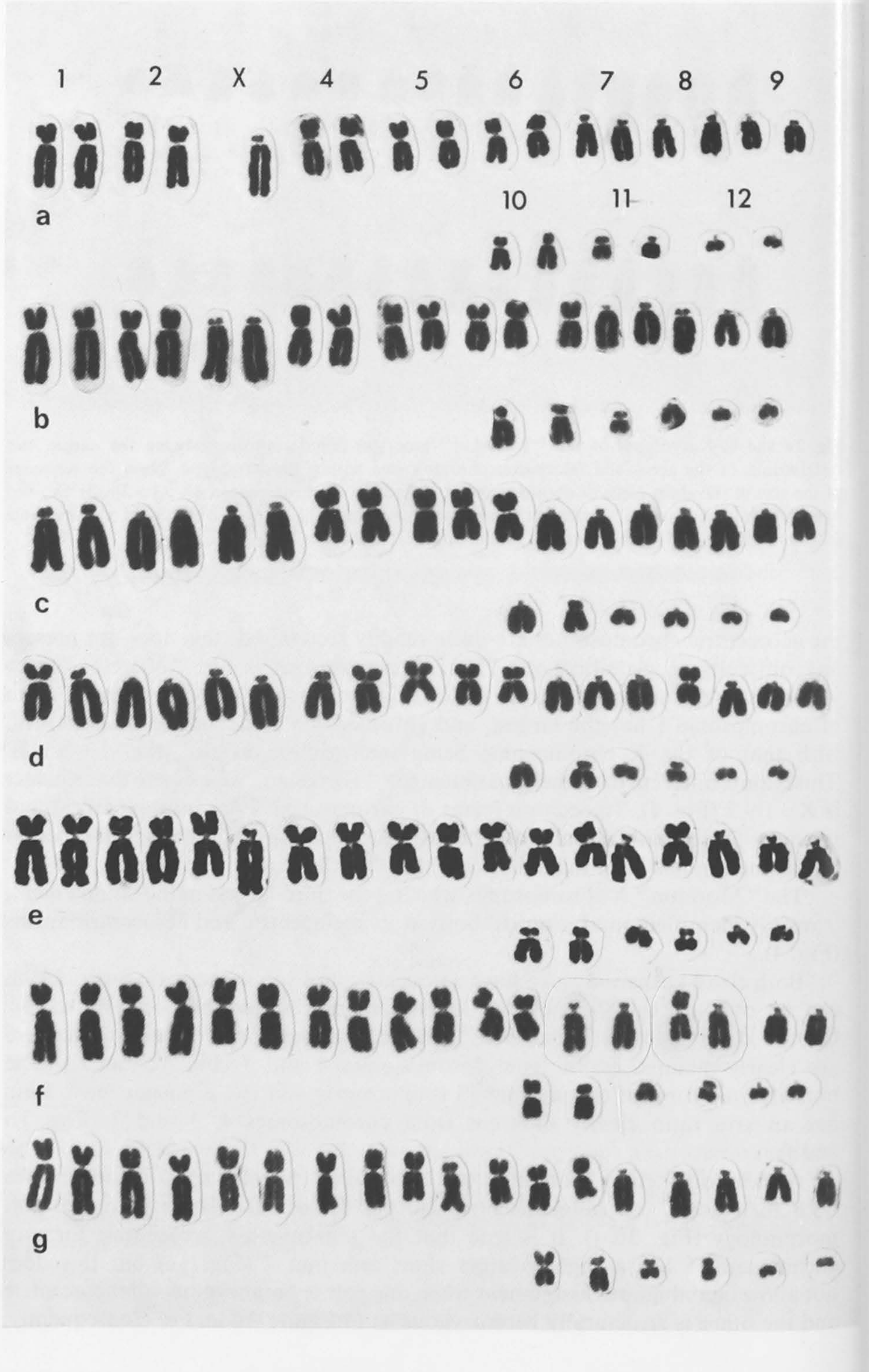

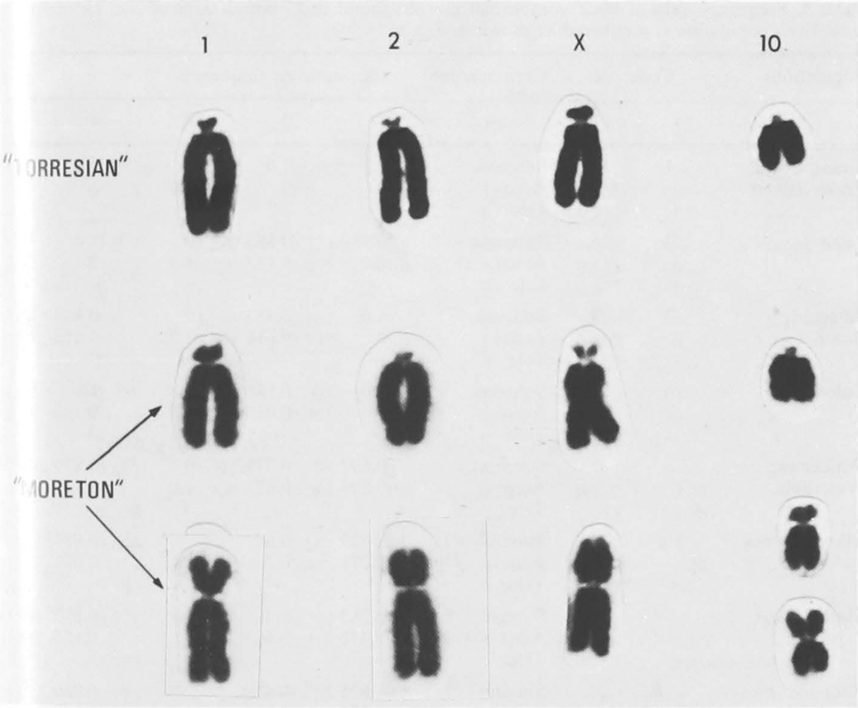

Fig. 4. The nature of the differences in shape of the acrocentric and metacentric autosomes (1, 2 and 10) and the X chromosome in the "Moreton" and "Torresian" races. In the "Torresian" race, the sizes of the short arms in chromosomes 1, 2 and X follow the sequence X > 1 > 2 and chromosome 10 is always telocentric. In the "Moreton" race, the sequence is 1 > X > 2 with chromosome 10 being telo-, acro- or metacentric

the frequency data for chromosomes 7 and 8 are not as accurate as those for the remaining members of the complement.

Chromosome 9 also exists in two forms; a telocentric which is structurally identical to the "Torresian" chromosome 9 (Fig. 3c) and an acrocentric form (Fig. 3g), although again there is some variability in expression of the short arm. For this reason no attempt has been made to distinguish the two types in frequency data (Table 3). A very rare metacentric form of this chromosome

Fig. 3a–g Representative karyotypes of the "Moreton" race. **a** Male mitosis of an individual form a coastal population. Autosomes 1–6 are homozygous submetacentrics associated with an acroecentric X chromosome, a characteristic feature of the coastal area. Chromosome 11 is heterozygous. **b** Female mitosis from a coastal population similar to **a** except that chromosome 7 is now present in the heterozygous condition. **c** Karyotype showing the acrocentric form of chromosomes 1, 2 and the X-chromosome. Note that in the "Moreton" race, the order of short arm size is now 1 > X > 2 (cf. Fig. 2a). Chromosomes 6 and 10 are present as heterozygotes. **d** Karyotype showing heterozygosity for chromosomes 1, 4, 6, 8, 10 and 11. **e** The X-chromosomes in this karyotype are now heterozygous, together with chromosomes 7, 8 and 11. **f** Female karyotype showing homozygosity for the submetacentric X-chromosome; heterozygosity for chromosomes 7 and 10. **g** Similar to (f) with the addition of heterozygosity for chromosomes 5, 7 and 10

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Table 3. Frequency data of the chromosomal morphs found in 27 populations of the "Moreton" race. Each population is numbered as shown in Figure 1

Populations	Code	N	Chromosome form	Chromosome frequency			
				1	2	X	4
Urang Creek, Fraser Island	1	18	Submeta	1.0	1.0	0	1.0
			Acro	0	0	1.0	0
			Telo	–	–	–	–
Tuan	2	26	Submeta	0.981	0.865	0	1.0
			Acro	0.019	0.135	1.0	0
			Telo	–	–	–	–
Peregian Beach	3	28	Submeta	1.0	0.982	0	0.982
			Acro	0	0.018	1.0	0.018
			Telo	–	–	–	–
Caloundra	4	37	Submeta	1.0	0.986	0	1.0
			Acro	0	0.014	1.0	0
			Telo	–	–	–	–
Walliebum Waterhole	5	33	Submeta	0.697	0.379	0	0.879
			Acro	0.303	0.621	1.0	0.121
			Telo	–	–	–	–
Maryborough	6	24	Submeta	0.729	0.625	0	0.937
			Acro	0.271	0.375	1.0	0.063
			Telo	–	–	–	–
Tiaro	7	24	Submeta	0.562	0.396	0	0.875
			Acro	0.438	0.604	1.0	0.125
			Telo	–	–	–	–
Redbank Creek	8	20	Submeta	0.625	0.425	0	0.950
			Acro	0.375	0.575	1.0	0.050
			Telo	–	–	–	–
Rosemount	9	19	Submeta	0.684	0.368	–	1.0
			Acro	0.316	0.632	1.0	0
			Telo	–	–	–	–
Spring Valley Creek	10	50	Submeta	0.46	0.28	0	0.88
			Acro	0.54	0.72	1.0	0.12
			Telo	–	–	–	–
Wahpunga Creek	11	21	Submeta	0.691	0.667	0.105	0.952
			Acro	0.301	0.333	0.895	0.048
			Telo	–	–	–	–
Coles Creek	12	47	Submeta	0.543	0.564	0.605	0.915
			Acro	0.457	0.436	0.395	0.085
			Telo	–	–	–	–
Cooroy	13	30	Submeta	0.800	0.783	0.205	0.933
			Acro	0.200	0.217	0.796	0.067
			Telo	–	–	–	–
Bald Knob	14	26	Submeta	0.635	0.712	0.429	0.885
			Acro	0.365	0.288	0.571	0.115
			Telo	–	–	–	–
Peachester	15	27	Submeta	0.778	0.759	0.333	0.982
			Acro	0.222	0.241	0.667	0.018
			Telo	–	–	–	–
Belli Creek	16	35	Submeta	0.714	0.543	0.630	0.943
			Acro	0.286	0.457	0.370	0.057
			Telo	–	–	–	–
Maleny	17	41	Submeta	0.781	0.866	0.983	0.963
			Acro	0.219	0.134	0.017	0.037
			Telo	–	–	–	–

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5	6	7	8	9	10	11	12	B
1.0	1.0	0.056	0	0	0	–	–	0
0	–	0.944	1.0	1.0	1.0	0.667	0	–
–	0	–	–	–	0	0.333	1.0	–
1.0	1.0	0.250	0.327	0	0	–	–	0.154
0	–	0.750	0.673	1.0	1.0	0.423	0	–
–	0	–	–	–	0	0.577	1.0	–
0.982	1.0	0.143	0.107	0	0	–	–	0.179
0.018	–	0.857	0.893	1.0	1.0	0.893	0	–
–	0	–	–	–	0	0.107	1.0	–
1.0	0.919	0.203	0.095	0	0	–	–	0.135
0	–	0.797	0.905	1.0	1.0	0.730	0	–
–	0.081	–	–	–	0	0.270	1.0	–
0.864	0.667	0.091	0.136	0	0	–	–	0
0.136	–	0.909	0.864	1.0	0.727	0.227	0.091	–
–	0.333	–	–	–	0.273	0.773	0.909	–
1.0	0.896	0.271	0.167	0	0	–	–	0
0	–	0.729	0.833	1.0	0.958	0.313	0.062	–
–	0.104	–	–	–	0.042	0.687	0.938	–
0.958	0.771	0.083	0.250	0	0.021	–	–	0
0.042	–	0.917	0.750	1.0	0.938	0.250	0.146	–
–	0.229	–	–	–	0.041	0.750	0.854	–
0.975	0.975	0.325	0.125	0	0	–	–	0
0.025	–	0.675	0.875	1.0	0.850	0.275	0	–
–	0.025	–	–	–	0.150	0.725	1.0	–
1.0	0.842	0.184	0.342	0	0	–	–	0
0	–	0.816	0.658	1.0	0.895	0.368	0.079	–
–	0.158	–	–	–	0.105	0.632	0.921	–
0.89	0.67	0.12	0.19	0	0	–	–	0.020
0.11	–	0.88	0.81	1.0	0.31	0.31	0.12	–
–	0.33	–	–	–	0.69	0.69	0.88	–
0.976	0.929	0.238	0.214	0	–	–	–	0
0.024	–	0.762	0.786	1.0	0.976	0.286	0.095	–
–	0.071	–	–	–	0.024	0.714	0.905	–
0.926	0.904	0.160	0.255	0	0	–	–	0
0.074	–	0.840	0.745	1.0	1.0	0.372	0.075	–
–	0.096	–	–	–	0	0.628	0.925	–
0.983	0.933	0.267	0.133	0	0	–	–	0
0.017	–	0.733	0.867	1.0	0.983	0.483	0.067	–
–	0.067	–	–	–	0.017	0.517	0.933	–
0.962	0.596	0.211	0.038	0	0	–	–	0.039
0.038	–	0.789	0.962	1.0	1.0	0.327	0.115	–
–	0.404	–	–	–	0	0.673	0.885	–
0.963	0.963	0.278	0.148	0	0	–	–	0.185
0.037	–	0.722	0.852	1.0	1.0	0.444	0.018	–
–	0.037	–	–	–	0	0.556	0.982	–
0.943	0.871	0.200	0.271	0	0	–	–	0
0.057	–	0.800	0.729	1.0	1.0	0.500	0.129	–
–	0.129	–	–	–	0	0.500	0.871	–
0.951	0.890	0.293	0.402	0	0.122	–	–	0
0.049	–	0.707	0.598	1.0	0.878	0.439	0.012	–
–	0.110	–	–	–	0	0.561	0.988	–

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Table 3 (continued)

Population	Code	N	Chromosome form	Chromosome frequency			
				1	2	X	4
Kenilworth	18	19	Submeta	0.816	0.658	1.0	1.0
			Acro	0.184	0.342	0	0
			Telo	–	–	–	–
Scrubby Creek	19	55	Submeta	0.727	0.782	1.0	0.991
			Acro	0.273	0.218	0	0.009
			Telo	–	–	–	–
Woodford	20	15	Submeta	0.833	0.90	1.0	0.967
			Acro	0.167	0.10	0	0.033
			Telo	–	–	–	–
Villeneuve	21	34	Submeta	0.75	0.912	0.982	0.882
			Acro	0.25	0.088	0.018	0.118
			Telo	–	–	–	–
Mt. Archer	22	22	Submeta	0.727	0.864	1.0	0.818
			Acro	0.273	0.136	0	0.182
			Telo	–	–	–	–
Oaky Creek	23	29	Submeta	0.897	0.741	1.0	0.810
			Acro	0.103	0.289	0	0.190
			Telo	–	–	–	–
Sheepstation Creek	24	32	Submeta	0.734	0.922	1.0	0.828
			Acro	0.266	0.078	0	0.172
			Telo	–	–	–	–
Kilcoy	25	33	Submeta	0.779	0.838	1.0	0.912
			Acro	0.221	0.162	0	0.088
			Telo	–	–	–	–
Transect 1 Population 5	26	30	Submeta	0.550	0.800	1.0	0.883
			Acro	0.450	0.200	0	0.117
			Telo	–	–	–	–
Transect 1 Population 4	27	31	Submeta	0.645	0.919	1.0	0.823
			Acro	0.355	0.081	0	0.177
			Telo	–	–	–	–

has also been found in the Mt. Archer population (Table 3, pop. 22). Likewise, chromosome 10 in the "Moreton" race may be either acro- (Fig. 3a–g) or telocentric (Fig. 3c and d), although it is most often found as an acrocentric. A metacentric form of this chromosome has also been found (Fig. 3f and g) and is generally associated with those populations carrying the submetacentric X chromosome.

Finally, the two smallest pairs of chromosomes in the complement (nos. 11 and 12) also have both acrocentric and telocentric morphs (Fig. 3). The acrocentric form of chromosome 12 is occasionally difficult to recognise because of its small size and it may have been underscored. (Fig 3e)

The Chromosomal Races and Their Eco-geographic Distribution

The "Torresian" race, previously referred to as the "General Purpose" race on account of its widespread distribution and characteristic Acridine karyotype, has been identified from southern Papua, Arnhem Land, Cape York Peninsula,

5	6	7	8	9	10	11	12	B
1.[illegible]	0.947	0.184	0.368	0	0	–	–	0
0	–	0.816	0.632	1.0	1.0	0.579	0.158	–
–	0.053	–	–	–	0	0.421	0.842	–
0.[illegible]55	0.909	0.245	0.264	0	0.118	–	–	0
0.[illegible]45	–	0.755	0.736	1.0	0.882	0.564	0.100	–
–	0.091	–	–	–	0	0.436	0.900	–
1.[illegible]	0.967	0.10	0.10	0	0	–	–	0
0	–	0.90	0.90	1.0	1.0	0.467	0	–
–	0.033	–	–	–	0	0.533	1.0	–
0.[illegible]97	0.956	0.412	0.338	0	0.279	–	–	0
0.[illegible]03	–	0.588	0.662	1.0	0.721	0.544	0	–
–	0.044	–	–	–	0	0.456	1.0	–
0.[illegible]64	0.955	0.136	0.318	0.023	0.341	–	–	0
0.[illegible]36	–	0.864	0.682	0.977	0.659	0.545	0	–
–	0.045	–	–	–	0	0.455	1.0	–
0.[illegible]93	0.948	0.086	0.379	0	0.310	–	–	0
0.207	–	0.914	0.621	1.0	0.690	0.690	0.017	–
–	0.052	–	–	–	0	0.310	0.983	–
0.844	0.922	0.281	0.250	0	0.250	–	–	0
0.156	–	0.719	0.750	1.0	0.688	0.547	0.016	–
–	0.078	–	–	–	0.062	0.453	0.984	–
0.824	0.912	0.191	0.309	0	0.353	–	–	0
0.176	–	0.809	0.691	1.0	0.603	0.471	0	–
–	0.088	–	–	–	0.044	0.529	1.0	–
0.800	0.900	0.150	0.267	0	0.233	–	–	0.033
0.200	–	0.850	0.733	1.0	0.733	0.500	0	–
–	0.100	–	–	–	0.034	0.500	1.0	–
0.919	0.935	0.274	0.274	0	0.355	–	–	0
0.081	–	0.726	0.726	1.0	0.580	0.484	0	–
–	0.065	–	–	–	0.065	0.516	1.0	–

down the east coast of Queensland as far south as Ipswich. The new name has been chosen to indicate its pattern of distribution. For the purpose of the work reported here, we shall refer only to samples taken in south-east Queensland, from Gin Gin in the north to Ipswich in the south, a distance of approximately 300 km.

The "Moreton" race, previously called the south-east Queensland race, has a far more restricted distribution and is associated with the coastal wallum and the inland region to the south and east of the Mary River. Its distribution extends from Maryborough and Fraser Island in the north as far south as Tyagarah in northern New South Wales. However, the major sampling reported here refers to the zone of about 100 km between Maryborough and Dayborough, to the north of Brisbane.

Figure 1 illustrates the distributions of both the Torresian and Moreton races in south-east Queensland and shows the locations of all sampling sites within the region. In no case, to date, have any sympatric populations been found even though the two races are separated by less than 1 km in at least four places, though this does not preclude their occurrence together over shorter distances. It is therefore apparent that these two races are parapatrically distributed and the estimated position of the line of parapatry is shown in Figure 5.

Since *Caledia* is fully winged, like other acridines, the narrowness of the boundary between the two chromosomal races is paradoxical. Parapatric distributions have never before been reported in acridine grasshoppers although they are common in the Morabines (Key, 1974) where the low mobility of these wingless insects is assumed to be one of the major factors contributing to the formation of the very narrow contact zone. This explanation may not be suitable for *Caledia* which is a strong flier and has an obvious capacity for dispersal. *Caledia* does not, however, show any annual or seasonal migratory patterns.

There are no obvious ecological parameters which can be correlated with the position of the tension zone, and the edaphic and vegetative characteristics of the region show considerable variation within the distributions of each race. However, one factor, which is not immediately obvious from field observations, is the pattern of seasonal variation found in this area. Climates – both past and present – are known to have played a crucial role in determining the distribution of much of the Australian flora and fauna (Burbidge, 1960; Keast, 1962) and seasonal moisture regimes are perhaps the most influential factors. The average weekly moisture index has been computed by Fitzpatrick and Nix (1973) from data collected from weather stations in southeast Queensland and maps have been prepared showing the summer (November-April) and winter (May-October) values. Only the winter iso-index lines correlate well with the patterns of distribution of the two chromosomal races of *Caledia*.

However, from a plot of the annual coefficient of variation around the mean weekly moisture index kindly provided by Henry Nix (CSIRO Land Use Research), we find that the iso-line representing 30% variation coincides, within the limits of precision of both lines, with the present day position of the tension zone between the two races (Fig. 5). The "Moreton" race is restricted to the region with less than 30% annual variation in weekly moisture index. Presumably the "Torresian" race would be expected to extend into the coastal areas with the lower level of climatic variation if that region were not already occupied by the "Moreton" race. It appears that seasonality of miosture may be a crucial factor in determining the position of equilibrium between the two races. This correlation becomes more significant if we follow the 30% variation contour to the west of Brisbane. The area around Toowoomba has a low variation level like that seen in the coastal region and here again we find that the populations of *Caledia* sampled from this area conform to the chromosomal characteristics of the "Moreton" race (Fig. 5).

One important factor associated with this climatic difference in racial habitat is seen in the number of generations per year. Thus the populations of the "Moreton" race have the capacity for at least two generations per annum whilst those of the "Torresian" race in S.E. Queensland are limited to one.

Karyotypic Variation within the "Moreton" Race

A detailed analysis has been made of the chromosomal variation within the "Moreton" race. Samples of approximately 30 individuals, including both males

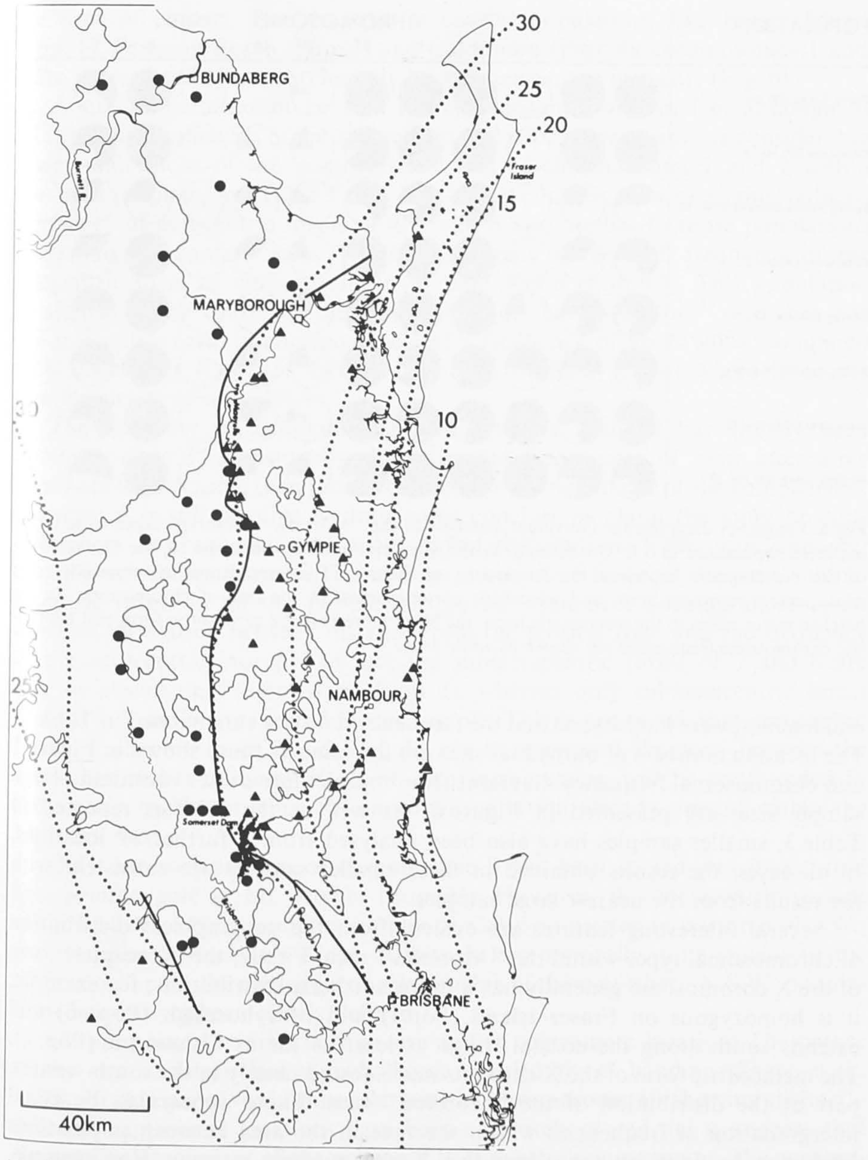

Fig. 5. The pattern of seasonal variation in the south-east Queensland region as measured by the coefficient of variation around the mean weekly moisture index estimated from data from 32 weather stations (H. Nix pers. comm.). The coastal zone shows the least variation in the moisture regime throughout the year presenting conditions for growth and development allowing 2–3 generations per annum of the "Moreton" race. To the west, the "Torresian" race occupies a much more variable environment, both in terms of moisture and thermal regimes and this race is univoltine. Note that, to the west of Brisbane, there is an area which has a similar moisture regime to the coastal zone and here, again, we find *C. captiva* with a similar karyotype to the "Moreton" race. It is interesting to note that an abrupt change in seasonality occurs in this region which coincides with the probable line of contact of the two races (solid line)

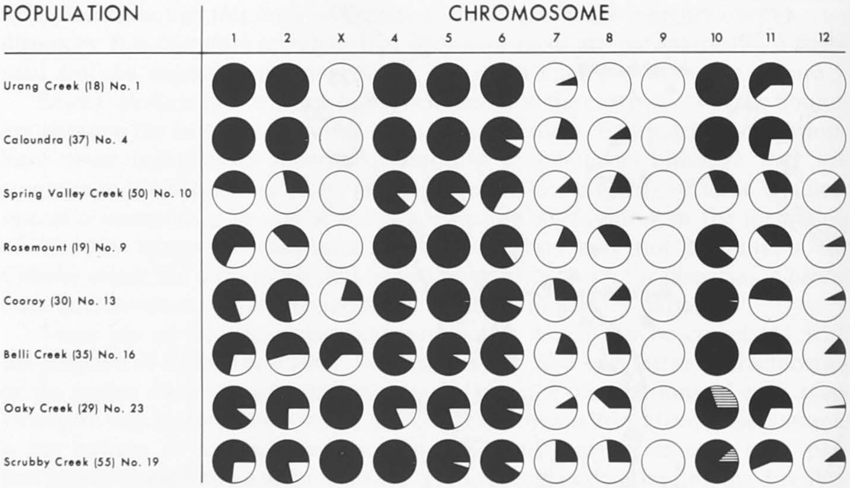

Fig. 6. Frequency diagrams for individual members of the "Moreton" karyotype taken from representative populations in S.E. Queensland. With the exception of chromosome 10, the black sectors of the pie diagrams represent the frequencies of meta- and submetacentric chromosomes; the white sectors represent acro- and telocentric chromosomes. In the case of chromosome 10, the hatched sector refers to the metacentric form, the black represents the acrocentric form and telocentric chromosome frequencies are shown as white areas

and females, were karyotyped and the frequency data are summarised in Table 3. The location numbers of individual sites are the same as those shown in Figure 1 and chromosomal frequency diagrams from representative sites containing large sample sizes are presented in Figure 6. As well as the 27 sites reported in Table 3, smaller samples have also been analysed from a further 17 localities. In all cases, the results obtained in these smaller samples are consistent with the results from the nearest large samples.

Several interesting features are evident from the geographical distribution of chromosomal types within the "Moreton" race. Firstly, the acrocentric form of the X chromosome generally has a more northerly distribution; for example, it is homozygous on Fraser Island (Pop.1) and Maryborough (Pop. 6) and extends south along the coastal fringe at least as far as Caloundra (Pop. 4). The metacentric form of the X chromosome is found mainly in the south-western part of the distribution of the "Moreton" race. There appears to be clinal intergradation of frequencies within the race in the area between populations known to be fixed on the alternative X chromosome morphs. For example, X chromosome frequencies at Wahpunga Creek (Pop. 11) and the intervening populations to the Maleny site (Pop. 17) show this clinal effect quite clearly (see Table 3 and Fig. 6).

The noninverted forms of chromosome 1 and 2 are most frequent in populations closest to the zone of contact between the "Moreton" and "Torresian" races. In general, there appears to be a gradual decrease in the frequencies of these noninverted chromosomal forms in populations furthest away from ie zone of contact. For example in coastal populations like Fraser Island 'op. 1), Peregian Beach (Pop. 3) and Caloundra (Pop. 4), chromosomes 1 and are represented almost exclusively by submetacentric elements (Fig. 6).

A similar pattern can be seen for chromosomes 4, 5 and 6, although in l cases the frequencies of the acrocentric and telocentric morphs is considerably wer than those of the noninverted forms of chromosomes 1 and 2 within e same population (Table 3 and Fig. 6). In contrast, however, this reduced equency of acrocentric forms of 4, 5 and 6 also applies to those populations osest to the contact zone. The only anomaly is the high frequency of the locentric form of chromosome 6 in Bald Knob (Pop. 14). This population almost in the centre of the distribution of the "Moreton" race, but has e telocentric form of chromosome 6 at a frequency of about 40%, compared ith Peachester (Pop. 15, 4 km to the west of Bald Knob) where it occurs a frequency of only 4%.

The difficulty in distinguishing chromosomes 7 and 8 has already been pointed out and consequently the estimated frequencies of their alternative forms are less accurate than those observed for the remainder of the complement. However it is still possible to draw some conclusions about the more obvious differences in frequency and geographical distribution. First, it is apparent that the acrocentric forms of 7 and 8 are much more frequent than the submetacentric forms in all the populations so far examined. Second, there is no obvious correlation between distance from the tension zone and the frequency of the acrocentric morphs. In fact the submetacentric forms of 7 and 8 are rare or absent on Fraser Island (Pop. 1), whereas only submetacentric forms of 1, 2, 4, 5 and 6 were found in this sample. This is not to say that acrocentric forms of chromosomes 7 and 8 could not have crossed the zone between the races, but rather that any effect of such introgression will be confounded in these cases by the primary chromosomal variation already present within the Moreton race.

Only one variant form of chromosome 9 has been scored and it occurred as a heterozygote at Mt. Archer (Pop. 22). However, this metacentric variant of the 9 has also been found in samples of *Caledia* collected in northern N.S.W., for example at Maclean's Ridge. Although karyotypically quite similar to the "Moreton" race, the phylogenetic relationship of these northern N.S.W. populations has not yet been clearly defined.

Chromosome 10 varies both in size and in centromere location. The nature of the size variation has already been revealed by C banding (Shaw et al., 1976). In this paper, only those variants of chromosome 10 showing distinctly different centromere locations have been accurately scored but it appears that the smaller size variant of chromosome 10 is relatively uncommon. The acrocentric 10, which is the most common and widely distributed, is present in all populations of the "Moreton" race and in many cases, particularly in the coastal region, it is the only form found. With the exception of one individual from Tiaro (Pop. 7) the metacentric form of chromosome 10 is restricted to the south western part of the distribution, and more specifically to those populations near fixation for the submetacentric X. In populations close to the contact zone between the races, a telocentric form of chromosome 10, morphologically

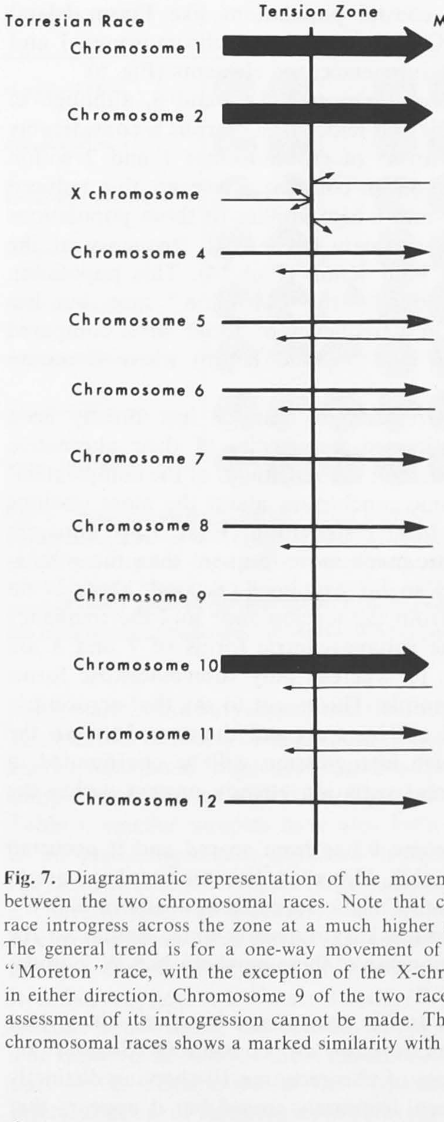

Fig. 7. Diagrammatic representation of the movement of chromosomes across the tension zone between the two chromosomal races. Note that chromosomes 1, 2 and 10 of the "Torresian" race introgress across the zone at a much higher frequency than the remainder of the genome. The general trend is for a one-way movement of chromosomes from the "Torresian" into the "Moreton" race, with the exception of the X-chromosomes which do not appear to introgress in either direction. Chromosome 9 of the two races cannot be accurately distinguished and thus assessment of its introgression cannot be made. The tension zone which exists between these two chromosomal races shows a marked similarity with the scheme proposed by Key (1974)

similar to the "Torresian" 10, is found (Fig. 4) often at quite high frequencies. For example, in Spring Valley Creek (Pop. 10) it occurs at a frequency of 69%.

The frequency data for chromosome 11 are similar to those for chromosomes 7 and 8, although in this case the data have been more accurately determined. On Fraser Island (Pop. 1), both the acrocentric and telocentric forms are present and no population in S.E. Queensland so far examined is fixed on either of the morphs. In general, however, the telocentric form of chromosome 11–

orphologically similar to the "Torresian" 11 – is most frequent in populations lose to the contact zone between the two races. For example, the telocentric 1 has a frequency of 69% in Spring Valley Creek (Pop. 10) but only 11% Peregian Beach (Pop. 3) 60 km to the S.E. of Spring Valley Creek.

The acrocentric form of chromosome 12 is difficult to recognise and is pparently rare. It was found to be absent from all the samples taken from e coastal populations.

Quite clearly, the karyotypic variation present within the "Moreton" race very complex but the nature of the variation, in terms of visible chromosomal patterning, allows us to draw several interesting conclusions.

Thus it is possible to recognise three major karyotypic regions within the Moreton" race: (1) the coastal populations, which are generally homozygous r the metacentric forms of chromosomes 1, 2, 4, 5 and 6 but also homozygous r the noninverted acrocentric X chromosome. (2) A south-western group, milar to the coastal types with one important exception. These populations e now fixed for the inverted metacentric form of the X chromosome. However, ere is evidence of autosomal introgression in populations collected near the ntact zone. (3) A northern group which, like the coastal group, carries the n-inverted X chromosome but also shows high levels of heterozygosity for romosomes 1, 2, 4, 5, 6 and 10, suggestive of chromosomal introgression from e "Torresian" race. Even so, from the very different frequencies of these romosomes, both within and between populations, it is evident that the rates of introgression are not the same for all chromosomes. Rather, we have a case of what Key (1974) has termed a "tension zone" in which chromosomes infiltrate across the zone differentially. Moreover, in the present case, chromosomes show both different frequencies along the contact zone and also differ in their degree of penetration into the "Moreton" race (Fig. 7). Thus the "Torresian" forms of chromosomes 2 and 10 have frequencies of 72% and 69% respectively at the contact zone (population 10) but 17.5 km to the north east (population 9) their frequencies change to 63% and 10.5% respectively. Clearly, there are important implications here regarding the evolutionary divergence and chromosomal interactions between the "Torresian" and "Moreton" races which warrants further investigation, particularly in those populations located along the "tension zone".

Discussion

The results presented here have revealed an unusually clear case of divergence involving major chromosomal rearrangement within a species. One, moreover, which involves the entire genome so that both racial identity and distribution pattern can be confidently determined. Furthermore, those samples collected along the zone of contact between the "Moreton" and "Torresian" races show that the level of hybridisation is limited both in its amount and in the width of the zone. Similar zones of contact between "races" or "species" of morabine grasshoppers have been emphasised by Key (1968) as "of crucial importance in any consideration of possible modes of speciation in these insects". Clearly

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this statement applies also to *Caledia* which shows a very similar pattern of contact to the pattern seen in morabines, in stick insects (Craddock, 1975), several mammalian species (Patton, 1969; Wahrman et al., 1969; Baker et al., 1976) and in the lizard *Sceloporus grammicus* (Hall and Selander, 1973). The recognition of these contact zones raises three important and, to date, unanswered questions regarding their function.

Firstly, how did the present day distribution patterns arise? Secondly, what are the evolutionary consequences of such zones of contact and thirdly, what role does genome reorganisation play in maintaining the integrity of the taxa concerned?

The first is still a contentious issue. White (1975) has proposed that, at least in the morabines, stick insects and some mammals, the chromosomal rearrangements arise initially within the [illegible] range of the parental distribution and lead to the production of adaptively superior homozygotes but inferior heterozygotes (stasipatric speciation). The perplexing problem here is that, at its initiation, the rearrangement is itself heterozygous and the probability of its survival must be very low since, in White's model, it would be in direct competition with the parental types. This stasipatric model of speciation in the morabines has been strongly challenged by Key (1974) on two grounds. First, there is no irrefutable evidence that the chromosomal rearrangements in the *viatica* group of morabines have produced good species since hybridisation studies offer no support for any marked reduction in viability or fertility in laboratory produced hybrids (Mrongovius, 1975). Secondly, the present day distribution patterns of the *viatica* group can be more logically interpreted as having arisen in allopatry, with subsequent secondary parapatry, rather than in sympatry.

In the case of the chromosomal races of *Caledia captiva,* where the past climatic history of the region they now occupy is well documented (Nix and Kalms, 1972; Kershaw, 1974; Shaw, 1976), the evidence would also support an allopatric origin. Furthermore, *Caledia* is not an isolated case of allopatric divergence in eastern Australia. Thus several species of birds, mammals and amphibians show distribution patterns very similar to that of *Caledia.* In the case of the fig parrots (*Opopsitta*), Keast (1960) recognised three distinctive isolates confined to each of three major tracts of rainforests along the east coast. The Rosella super species *Platycercus eximius* has two species (*P. adscitus* and *P. eximius*) located in north-east and south-east Australia respectively. These two species have made secondary contact with limited hybridisation in the south-east Queensland region. Other examples have been cited by Keast (1962) and there is no doubt that past climatic upheavals in this area have produced conditions resulting in the isolation of species in "refugia" areas, which in some cases has led to subsequent allopatric divergence. A particularly relevant example is seen in the case of the "acid" frogs which inhabit the wallum of south-eastern Queensland (Straughn and Main, 1966; Ingram and Corben, 1975). Several cases of parapatry between "acid" and non-wallum species of the genera *Litoria* and *Ranidella* (*Crinia*) are known, and the geographical distribution of several species of these genera bears a striking resemblance to the situation in *Caledia.* Ingram and Corben postulate that the "acid" species, which live in water with a pH of 4.3–5.2, have evolved in isolation in the allum, which provided a moist environment during the dry Pleistocene interplu- ials when the surrounding areas would be uninhabitable to other frog species. ll the non-wallum species which are now in close contact with the "acid" pecies are considered to have had a southern origin and have since expanded orth. In *Caledia,* it is proposed that the "Torresian" race is of a northern rigin, possibly southern Papua or Arnhem Land and the zone of "secondary ntergradation" (Mayr, 1963) has arisen through climatic change, possibly coupled ith agricultural development, this having occurred relatively recently in evolu- onary terms. Since the establishment of secondary contact, chromosomal intro- ression has occurred between the races (Figs. 6 and 7). This can be deduced om the pattern and frequency of chromosomal morphs within the "Moreton" ace. Some chromosomes, such as 1, 2 and 10 appear to have introgressed a high frequency and with high penetrance, from the "Torresian" into the Moreton" race. Other chromosomes, such as 4, 5 and 6, have done so at much lower rate as seen from their lower frequencies and degree of penetrance. utosomes of the "Moreton" race only very rarely manage to introgress into e "Torresian" race. This can probably be attributed to the unfavourable cological conditions and genetic background in which the "foreign" chromo- omes find themselves. The X chromosomes do not appear to introgress in ither direction. It would be premature, at this stage, to propose a mechanism esponsible for chromosome elimination in "Torresian" × "Moreton" hybrids ntil we have acquired more data from within the zone of contact. The present ay ecological associations of these two races suggest that the "Moreton" ace would extend its range extremely slowly into those unfavourable areas o the west, if the "Torresian" race was not already present. Reciprocally, he latter would rapidly expand into the "Moreton" habitat if it were not already occupied by a better adapted form.

C-banding* studies (Shaw et al., 1976) have revealed that the acrocentric and telocentric chromosomes within the "Moreton" race have banding patterns similar to their submetacentric homologues. This appears to be an immediate objection to the proposed hypothesis of chromosomal introgression. The "Torresian" chromosomes have only small centric C-banded regions whilst both centric and interstitial bands are present on the "Moreton" chromosomes. However, a simple scheme of crossing over in F_1 hybrids, coupled with backcrossing would explain the presence of C-bands on the acro- and telocentric morphs in the "Moreton" race. More importantly, it would explain the polymorphisms for the C-bands that Shaw et al. reported for virtually all of the autosomes and, similarly, the absence of any C-band polymorphism for the X chromosome. Furthermore, the presence of acro- and telocentric chromosomes in the "Moreton" race may give the illusion of movement of whole chromosomes across the contact zone. In fact, it may well be that, in general, only that portion of the acrocentric chromosomes, corresponding to the shorter arms of the submetacentrics (23–44%), which is not involved in crossing over, manages to maintain its unity in the "foreign" genetic background. In other words, when the F_1 hybrid backcrosses to the parental "Moreton" population there appears to be strong selection for gametes which most closely resemble those of the parental population in gene and C-band pattern.

* (See also Chapter I,p17)

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Our interpretation of the formation of the “tension zone” between these two chromosomal races is very similar to that proposed by Key (1974) in which, initially, chromosomal rearrangements reach fixation in allopatry and subsequent expansion gives secondary parapatric contact. What happens along such a region of contiguity is mainly speculative at the present time since the relevant studies have not been made, but it is clear that the consequences are dependent upon the degree of genetic divergence which arose in allopatry. Basically there are three options; (1) if genetic divergence is complete then the two taxa now represent good species and could co-exist in sympatry; (2) one “race” may completely displace the other resulting in extinction of the less fit “race”; (3) if hybridisation occurs between the two races then here the consequences depend mainly on the fitness of the heterozygote. If hybrid fitness is not impaired in any way then eventually the two races should fuse together to produce a widespread polymorphic situation. In cases of reduced hybrid fitness, one might expect that the intensity of the selection for premating isolation would be proportional to the reduction in fitness of the hybrid. This would also tend to limit the width of the hybrid zone. In the case of *Caledia captiva* it is quite clear that, in the main, option 3 applies, although with certain modifications to the scheme proposed by Key. On evidence deduced from the morabines, Key proposes that the line of contact between two races is too smooth to result from ecological tolerance differences, although there is no good evidence to support this view since no hybrid zone has been studied in sufficient detail. We maintain that the position of the contact zone between the two races of *Caledia* is probably a result of an interaction between both ecological tolerance and competitive exclusion. Our argument is based on three facts. First, the centromeric rearrangements which have led to genome reorganisation between the parapatric races would not result in any instability in hybrids due solely to mechanical problems during meiosis. For the same reason, in our case a stasipatric mechanism of the kind envisaged by White could not apply. Secondly, the position of the tension zone coincides remarkably well with a change in climatic and ecological conditions in terms of both the amount and the variability in rainfall and in seasonsality. Thus the “Moreton” race occupies a more favourable environment which permits at least 2 generations a year whereas the “Torresian” race to the west occupies territory subjected to much drier summers and colder winters restricting it to only one generation per year. Thirdly, evidence from introgression clearly indicates that chromosomes are moving in one direction only, form the “Torresian” into the “Moreton” race (Fig. 6). Moreover, introgression involves only a limited part of the genome with rapid elimination of the residual “foreign” chromosomes.

Clearly, any meaningful interpretation of the level of genic differentiation and divergence between these two chromosomal races cannot be logically made from the present data, as is also the case in all other known examples of parapatric association of chromosomally different taxa. Information on the level of genetic divergence must be otained before any assessment can be made of the role of the chromosomal rearrangements in maintaining racial integrity. We consider that the case of *Caledia captiva* presents an ideal opportunity for such an assessment.

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CHAPTER III

The Hybrid Zone

INTRODUCTION

Hybrid zones, recognizable on morphological or chromosomal criteria, have been found in many groups of invertebrates and vertebrates (Mayr, 1963; Short, 1972; Chapter II). The status of the "populations" on either side of these hybrid zones has invariably aroused taxonomical controversy, to the extent that Bigelow (1965) proposed that "the interpretation of hybrid zones remains one of the most difficult of taxonomic problems, despite the striking advances in systematics of recent decades". The advances referred to are principally the development of the biological species concept in which sexual isolation, hybrid infertility and developmental incompatibility are the main criteria for determining the specific status of particular taxa. However all of these criteria may break down within hybrid zones.

The most frequently cited species definition is that of Mayr (1963), which states that "species are groups of actually (or potentially) interbreeding natural populations which are reproductively isolated from other such groups". Bigelow is highly critical of the term "interbreeding" in this definition since "hybridization does not always tend to make two gene pools progressively more similar, even when the hybrids are fertile". The long term stability of several hybrid zones including that in the carrion crow, which is discussed herein, demonstrates this point.

Consequently, Bigelow (1965) redefined a species as "a group of natural populations that is reproductively isolated from other such groups, but in which the component populations are not reproductively isolated from one another. Reproductive isolation should be considered in terms of gene flow and not in terms of interbreeding, since selection will inhibit gene flow between two well integrated gene pools despite interbreeding". However precisely the same criticism can be applied to this definition that Bigelow himself applied to Mayr's. Lack of gene flow, like the absence of interbreeding, is not an absolute criterion of species status, otherwise it would not be necessary to define introgression as the incorporation of genes of one species into the gene pool of another. Therefore the presence or absence of gene flow cannot be the basis of an operational species definition. This is because those genes or chromosomes which do not disrupt the well integrated harmony of another gene pool, by adverse heterotic or epistatic effects, will be incorporated into the foreign gene pool, provided they can overcome the barrier of selection acting against the other non-harmonious combinations in the backcross generations. Thus introgression of favourable genes will be possible if such genes can be separated by segregation and recombination from those genes which are incompatible with the foreign gene pool. The existence of coadapted gene pools does not preclude the possibility that some genes or chromosomal segments will perform equally well in either genetic background and hence will enhance the genetic variation of the recipient species. Indeed it is feasible that such introgressed genes can, in fact, raise the general fitness of the recipient species. Thus introgessed populations of *Drosophila mojavensis*, containing genetic material from *D. arizonensis*, have been shown to be significantly fitter on the basis of several fitness measures than either of the parental forms (Nagle and Mettler, 1969). In this case, the experimental introduction of genetic material has increased the fitness of the introgressed species.

Even so, in the majority of cases, the existence of a hybrid zone is taken as evidence for the existence of reproductive isolation between two taxa. A detailed analysis of the genetic structure of a hybrid zone can be expected to reveal, at least in part, some of the mechanisms involved in this isolation. Very few such studies exist due to problems in precisely locating the contact zone between the taxa and because of intrinsic difficulties in obtaining adequate samples to allow a meaningful analysis to be made. The hybrid zone between the Carrion crow *Corvus corone* and the Hooded crow *C. cornix* is a classic example of a stable hybrid zone and is one of the few cases to be analysed in detail (Mayr, 1963). The zone is 75 to 100 kilometres wide, extending from Scotland, through Denmark and Central Europe to the Mediterranean. Within the hybrid zone, mating is apparently at random and the nests contain normal numbers of progeny. The hybrids are considered to be fertile and every conceivable combination of parental characters occur within the zone. Introgressed characters are occasionally found outside the zone, but in general the two species maintain their integrity on either side of it. Behavioural isolation and hybrid sterility can, in this case, be eliminated as factors responsible for the maintenance of the distinction between these species, although it is still not known which mechanisms are responsible for the isolation.

Where hybrids are determined on chromosomal criteria, it may be impossible to distinguish between F1 hybrids and backcrosses, unless the two taxa show multiple karyotypic differences. Therefore the high level of chromosomal differentiation found between the "Moreton" and "Torresian" races of *C. captiva* makes the hybrid zone between them particularly suitable for a comprehensive analysis to be made. The large number of karyotypic differences between the parental forms permits an assessment of the possible restrictions on both the number and type of backcross and F2 progeny, providing an indication of, and means of distinguishing between, hybrid sterility or hybrid breakdown.

One major difficulty and one, moreover, which is critical in studying a hybrid zone is the problem of obtaining adequate sample sizes, which permit a meaningful analysis to be made. This is well illustrated in the study of the hybrid zones between chromosomal races of the mole rat, *Spalax ehrenbergii* (Nevo and Bar-El, 1976). Four parapatric races of this species with 52, 54, 58 and 60 chromosomes are found in Israel. Hardy-Weinberg conditions are satisfied in the 58-60 contact zone, implying random mating between and within the races. However these conditions are not satisfied in the 54-58 and 52-58 zones, which are much narrower than the 58-60 zone. The authors interpret the disequilibrium and the more extreme narrowness of the latter two zones as evidence for enhanced reproductive isolation. However the apparent deficit of hybrids in these two zones may be a reflection of inadequate sampling in an area which covers too large a proportion of the zone. Because the racial frequencies change abruptly as a steep cline across the zone, any sampling strategy which covers too large a part of this cline will necessarily give an apparent deficit of hybrids. Since heterogeneous racial frequencies will be unknowingly pooled, the hybrid deficit will be an expression of the "Wahlund" effect rather than of the breeding structure of the populations in the hybrid zone. In the *Spalax* case, the zones with apparent hybrid deficits are also the narrower zones in which such deficits would be more likely to result from the "Wahlund" effect. Adequate sampling at restricted points across the zone is therefore essential for the meaningful analysis of the breeding structure of any hybrid zone. In the case of the parapatric races of *Caledia* in south east Queensland, the population densities in the vicinity of the hybrid zone are sufficiently high to allow a comprehensive collection of samples to be made. Population densities in this region have been estimated as 3,000 adult individuals per hectare (Craft, pers. comm.) and furthermore the distribution of grasshoppers across the zone is uninterrupted by geographical barriers.

The Location of the Transects

Two transects have been made, one between a "Torresian" and a "Moreton" metacentric X population and the other between a "Torresian" and a "Moreton" acrocentric X population, in order to locate precisely the position of the hybrid zone and ultimately to analyse its structure.

Transect 1 lies between the Gregors Creek "Torresian" population and the Kilcoy "Moreton" metacentric X population (see Chapter II). Transect 2, on the other hand, was made between the Bongmuller Creek "Torresian" population and the Spring Valley Creek "Moreton" acrocentric X population. In both cases, the zone of contact was located to within a kilometre. It was not possible to sample Transect 2 in finer detail, because the Mary River lies between the two nearest "Torresian" and "Moreton" populations, at Bell's Bridge and Spring Valley Creek respectively. However no geographical barrier exists in Transect 1 and it was possible to continue detailed sampling of the 1 kilometre interval. In neither case is the position of contact associated with any abrupt ecological change, but there is a gradual increase in aridity and seasonality in a westward direction between the races. Although the Mary River runs across Transect 2 and separates the nearest "Torresian" and "Moreton" populations, sampling at other localities on either side of the river (see Chapter II) indicates that it is not a primary geographical barrier separating the races in this region. In any case, Transect 2 is not discussed in detail in this Chapter since racially mixed populations were not sampled and the structure of the hybrid zone therefore could not be analyzed.

MATERIALS AND METHODS

The position of the contact zone was located using a sequence of transects with progressively smaller intervals. Originally a 20 km transect, with 5 km intervals, was made in an easterly direction from the Gregors Creek site (T1P1) towards Kilcoy. The racial changeover was found to occur between the T1P3 and T1P4 sites. Subsequent sampling at 1 km intervals (A,B,C,D) between T1P3 and T1P4, revealed that the T1PA sample was "Torresian" and the T1PB sample "Moreton" in constitution.

In January 1977, at a time of likely maximal population density, a 200 metre interval transect (A1,A2,A3,A4) was made between T1PA and T1PB (Figure 3.1). T1PA was resampled since only a small number of individuals had been previously collected from this site. Collecting

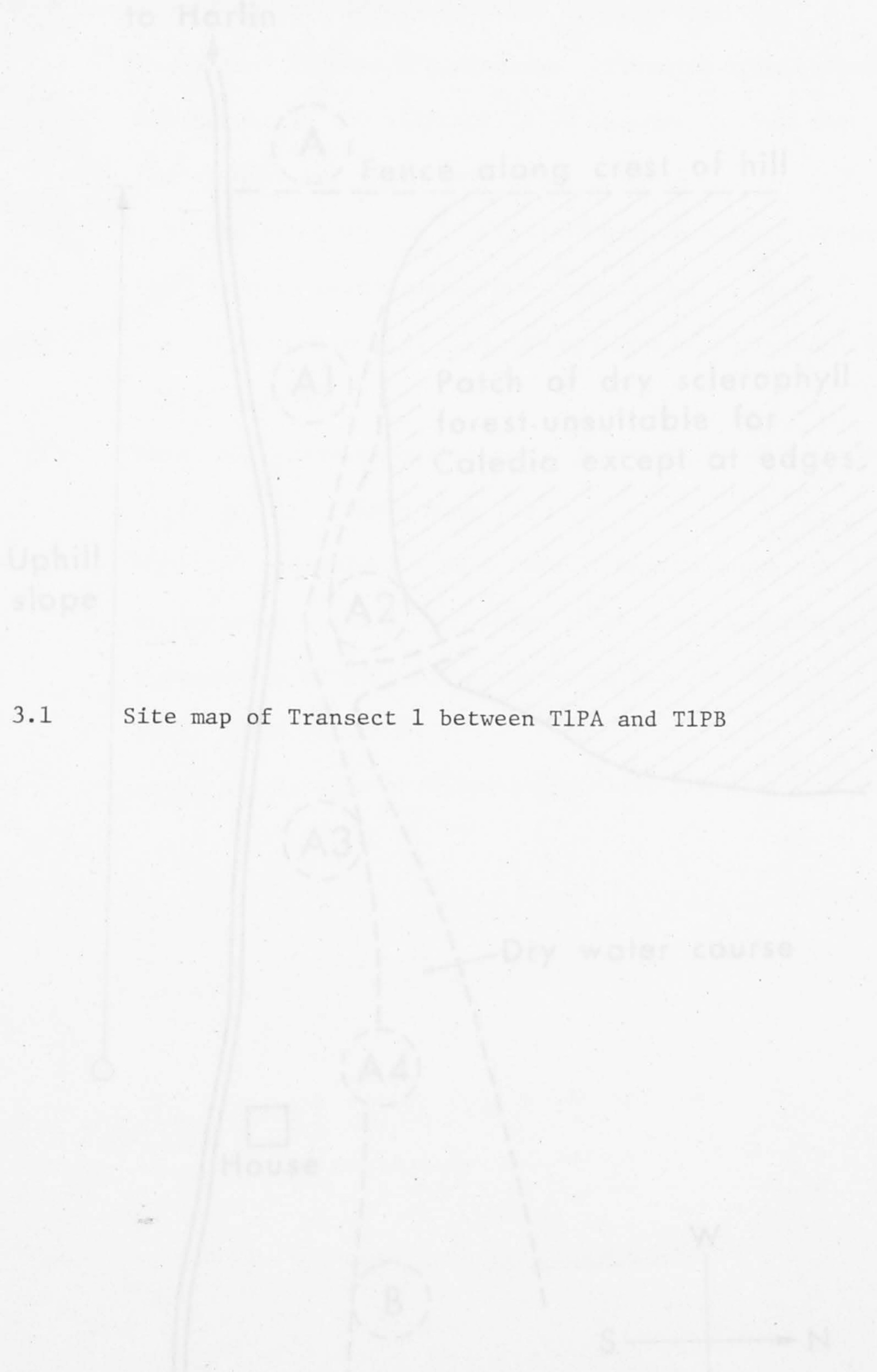


Figure 3.1 Site map of Transect 1 between T1PA and T1PB

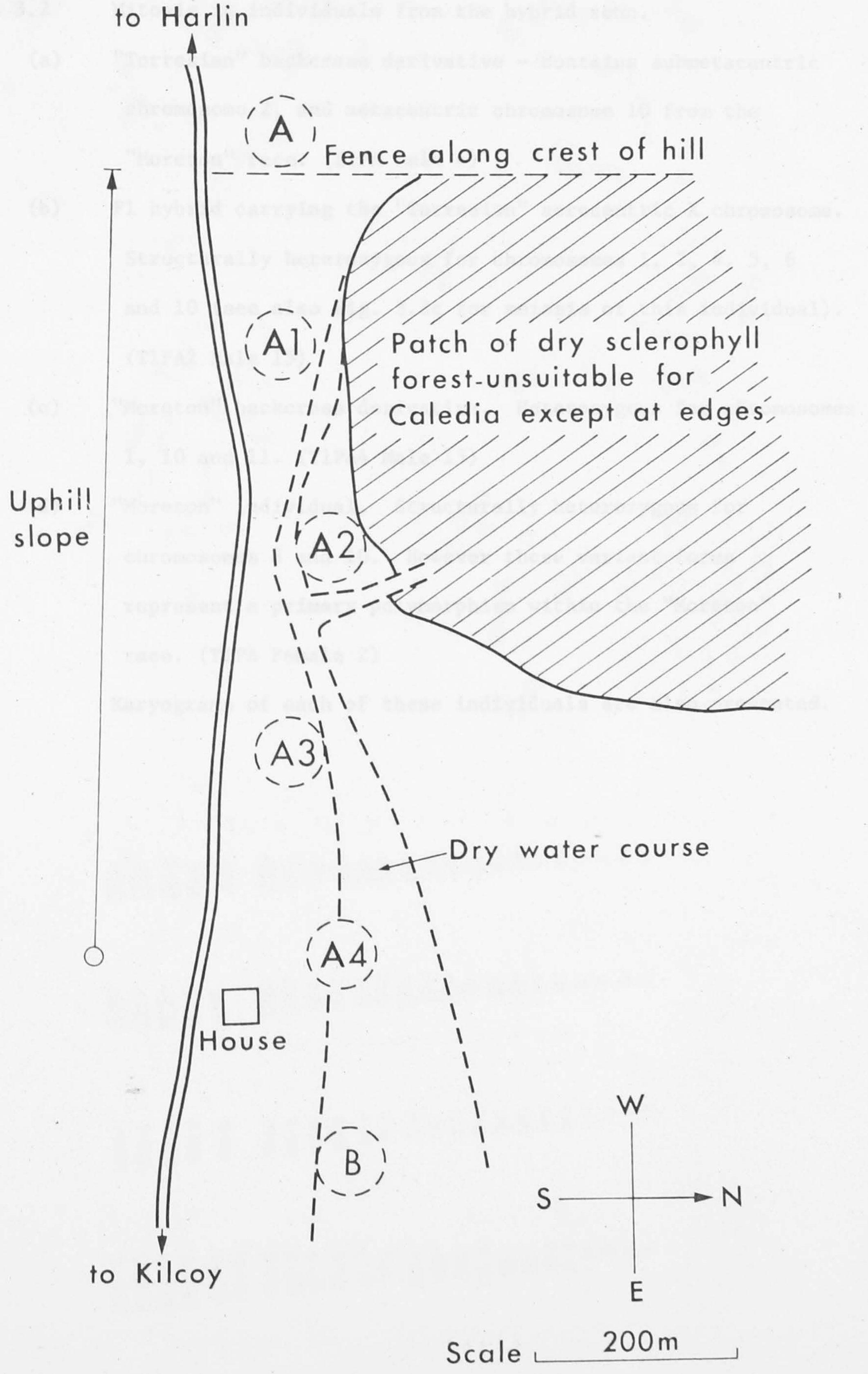

to Harlin
A
Fence along crest of hill
A1
Patch of dry sclerophyll forest-unsuitable for Caledia except at edges
Uphill slope
A2
A3
Dry water course
A4
House
B
W
S
N
E
to Kilcoy
Scale 200m

Figure 3.2 Mitosis in individuals from the hybrid zone.

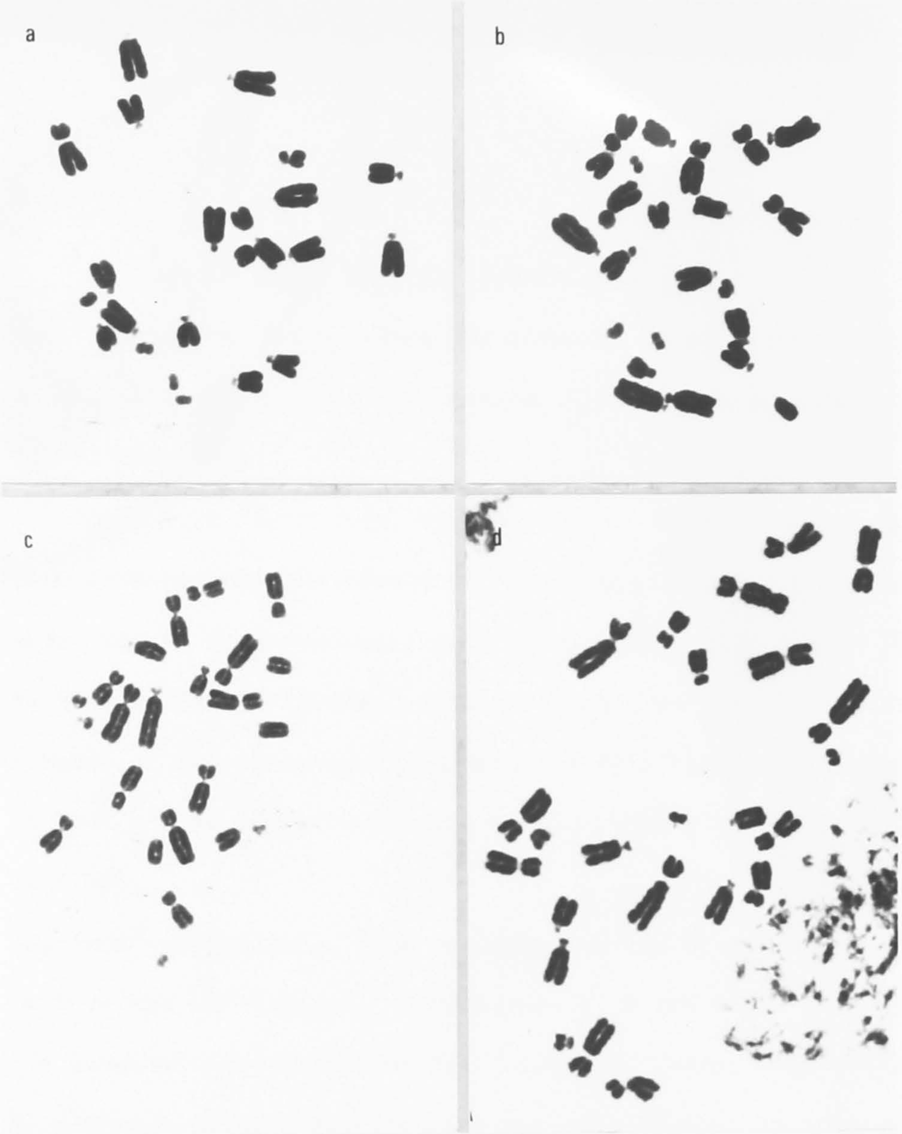
(a) "Torresian" backcross derivative - contains submetacentric chromosome 2, and metacentric chromosome 10 from the "Moreton" race. (T1PA Male 4)

(b) F1 hybrid carrying the "Torresian" acrocentric X chromosome. Structurally heterozygous for chromosomes 1, 2, 4, 5, 6 and 10 (see also Fig. 3.3c for meiosis of this individual). (T1PA2 Male 15)

(c) "Moreton" backcross derivative. Heterozygous for chromosomes 1, 10 and 11. (T1PA4 Male 15)

(d) "Moreton" individual. Structurally heterozygous for chromosomes 8 and 10. However these variant forms represent a primary polymorphism within the "Moreton" race. (T1PB Female 2)

Karyograms of each of these individuals are also presented.


a
b
c
d

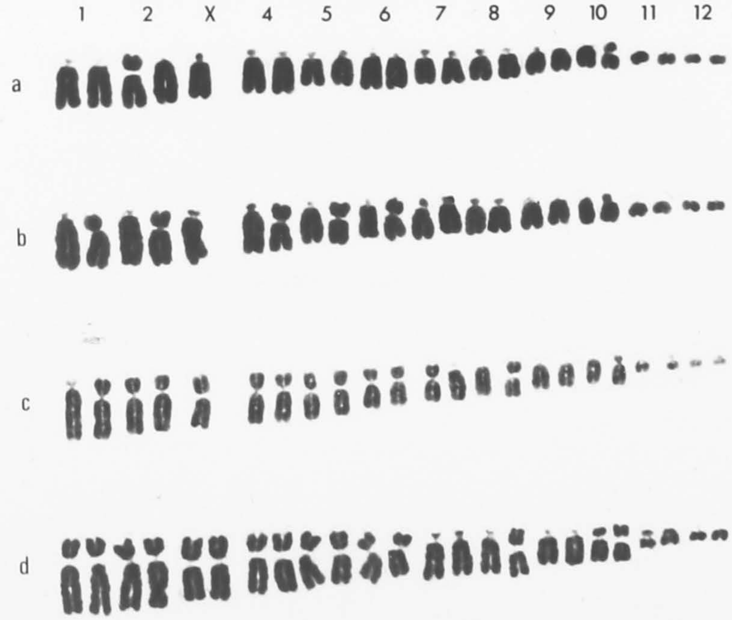

1
2
X
4
5
6
7
8
9
10
11
12
a
b
c
d

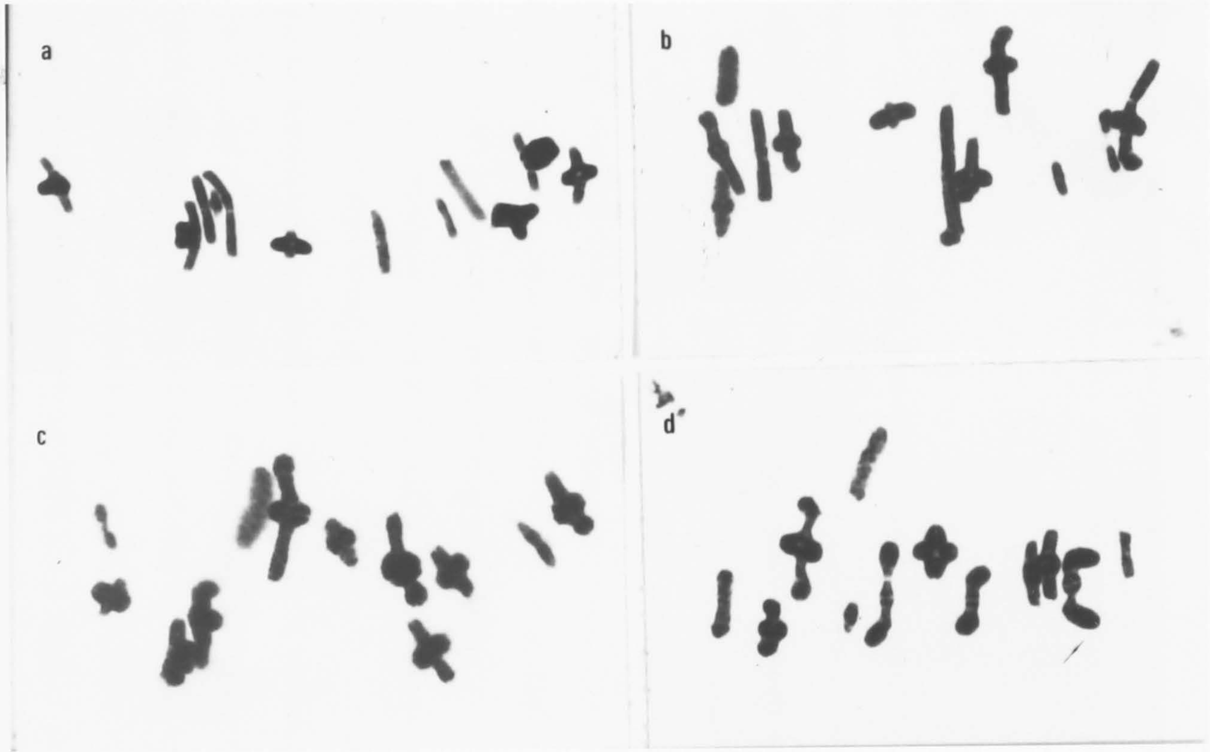
Figure 3.3 Meiosis in males from the hybrid zone.

(a) Pure "Torresian" male. Note the terminal or subterminal position of all centromeres and the presence of interstitial chiasmata. (T1PA1 Male 4)

(b) F1 hybrid with "Torresian" acrocentric X. Note the heteromorphic structure of 6 of the bivalents, resulting from flexure of the short arm of the submetracentric chromosomes. (T1PA2 Male 16)

(c) F1 hybrid with "Torresian" acrocentric X (see also Figure 3.2). Flexure of the short arm of submetracentric chromosomes apparent in five of the six structurally heterozygous bivalents. (T1PA2 Male 15).

(d) "Moreton" individual. Note homozygosity for flexed short arms in 6 of the chromosomes. Chromosomes 7, 8 and 9 are homozygous for acrocentric or telocentric chromosomal forms. The heteropycnotic X, although submetacentric, does not show flexure in this cell, although this is often observed in other cells from the same individual. (T1PA3 Male 3).


a
b
c
d

was restricted to a radius of about 20 metres around the end point of each 200 metre interval. About 40 individuals were collected at each point.

The analysis of karyotypes was carried out essentially as reported in Chapter II. However pure "Torresian" karyotypes were not photographed. Karyograms were constructed for all "Torresian" individuals bearing inversions and all "Moreton" individuals to allow precise chromosome identification. Testis material was also fixed from all males sampled from the hybrid zone, so that meiosis could be examined in the F1 hybrids (see Chapter V).

OBSERVATIONS

1. *The number of inversions per individual in Transect 1*

The average number of inverted chromosomes per individual, together with the standard deviation, have been calculated for each of the samples from the transect (Table 3.1). These values have been considered separately for males and females because of the difference in chromosome number resulting from the XO/XX sex chromosome system. Precise chromosome identification is not required for this method of analysis, but simply a classification into two unambiguous categories. An analysis involving individual chromosome identification will be presented in subsequent sections of this chapter.

For a pure "Torresian" population, the number of inversions per individual is 0. For a pure "Moreton" population, it is more difficult to estimate this parameter because of the primary polymorphism for autosomes 7, 8 and 11. However for a pure population fixed for the metacentric form of the X chromsome, the individual values range from 16 to 19 for males and 17 to 20 for females, allowing for this primary polymorphism.

An analysis of the difference in the mean number of inversions per individual has been carried out on adjacent populations in the transect. The major change in the mean number of inversions per individual occurs between the samples from T1PA2 and T1PA3 (Table 3.1).

TABLE 3.1 Number of "inversions" per individual

	Male data			Female data		
Population	Mean	S.D.	N	Mean	S.D.	N
T1P3	0.12	0.33	17	0.05	0.22	21
T1PA	0.85	0.93	20	0.70	0.76	23
T1PA1	3.00	4.23	15	2.32	2.94	25
T1PA2	3.27	3.88	22	2.28	3.51	18
T1PA3	10.33	5.03	15	12.89	3.21	27
T1PA4	13.55	1.64	20	13.09	2.17	23
T1PB	14.56	2.13	9	14.29	1.21	17
T1P4	12.92	1.44	13	14.50	1.46	18

TABLE 3.2	Male data			Female data		
Comparison	t	D.F.	P	t	D.F.	P
3-A	3.276	24*	<.01	3.925	26*	<<.01
A-A1	1.870	15*	N.S.	2.660	27*	<.05
A1-A2	0.200	35	N.S.	0.041	41	N.S.
A2-A3	4.818	35	<<.01	10.465	43	<<.01
A3-A4	2.386	16*	<.05	1.567	45*	N.S.
A4-B	1.399	27	N.S.	2.225	36*	<.05
B-4	2.162	20	<.05	0.462	33	N.S.

Pairwise analysis of the differences between adjacent populations by means of "Students" t test

* Variances are signficantly different by variance ratio F test. Modified t test (Bailey, 1959) has been applied.

These differences are very highly significant for both males and females (Table 3.2). Few of the changes in this parameter in the remaining comparisons between adjacent samples are significant, although there are signficant and consistent changes for both sexes between T1P3 and T1PA (Tables 3.1, 3.2). These are predominantly "Torresian" populations, which are 1 kilometre apart. This result suggests that selection is still acting strongly against individual "Moreton" chromosomes in the "Torresian" genetic background since neither of these populations is racially mixed or contains F1 hybrids. However, there is no evidence of similar selection acting against single chromosomes between T1PB and T1P4, which are 3 km apart on the "Moreton" side of the zone. Again neither population is racially mixed nor contains F1 hybrids, but there is not a statistically significant increase in the average number of inversions per individual as would be expected if selection were acting against individual foreign autosomes. Indeed the male data, if anything, indicate a significant trend in the other direction (Tables 3.1, 3.2) between T1PB and T1P4, although this decrease is only marginally significant and not present in the females. Thus if selection is acting against the "Torresian" autosomes in the "Moreton" background, it is much less severe than the selection against individual "Moreton" chromosomes on the "Torresian" side of the zone.

This interpretation of differential selection is supported by a comparison of the observed average number of inversions in samples from either side of the hybrid zone. For example in T1P3, which is only 1.5 km from the centre of the zone, the observed number of inversions (Table 3.1) is very close to the expected value of 0 for a pure "Torresian" population. On the other hand, on the "Moreton" side of the zone at T1P4, the observed average number of inversions (Table 3.1) is about 3 less than even the minimal expected value of 16 or 17, even though this locality is 3.5 km from the centre. Therefore "Moreton" chromosomes are rapidly eliminated on the "Torresian" side of the zone, whereas the "Torresian" autosomes persist on the "Moreton" side. This evidence further supports the interpretation made in Chapter II, that a considerable amount of the chromosomal variability in the "Moreton" race has arisen by one way movement of "Torresian" chromosomes into the "Moreton" race.

2. *F1 Hybrids and Parental Types in the Contact Zone*

The individuals from each sample across the transect have been classified into three categories (see Figures 3.2 and 3.3 for examples). These are:-

(i) "Torresian" (pure and backcross derivatives)

(ii) F1 hybrids

(iii) "Moreton" (pure and backcross derivatives)

The diagnostic characteristic of F1 hybrids is, of course, maximal chromosomal heterozygosity. However because backcross individuals are frequent, some allowance must be made for F1 type individuals derived by hybridization between "introgressed" individuals of the two types. Hybrids derived from such matings will be homozygous for one or more diagnostic chromosomal pairs. For example, in T1PA1, 10 of the 13 "Torresian" males had one or more inverted chromosomes, with an average of 1.9 inversions per backcross derived individual. Similarly, 14 of the 22 "Torresian" females from the same sample had an average of 2.3 inversions per individual. Similar statistics for the other predominantly "Torresian" populations are presented in Table 3.3. Consequently a small proportion of the individuals classified as backcross derivatives may in fact be F1 hybrids and vice versa. In practice, however, the proportion of such ambiguous individuals is very low and unlikely to affect the usefulness of the data. It is more difficult to assess this effect on the "Moreton" side of the zone, partly because of the primary polymorphism for chromosomes 7, 8 and 11 and partly because there are fewer restrictions on the occurrence of new segregants on the "Moreton" side of the zone as a subsequent section will demonstrate.

The racial frequencies, based on total genome classification, show

TABLE 3.3

Average number of inversions in "Torresian" backcross individuals

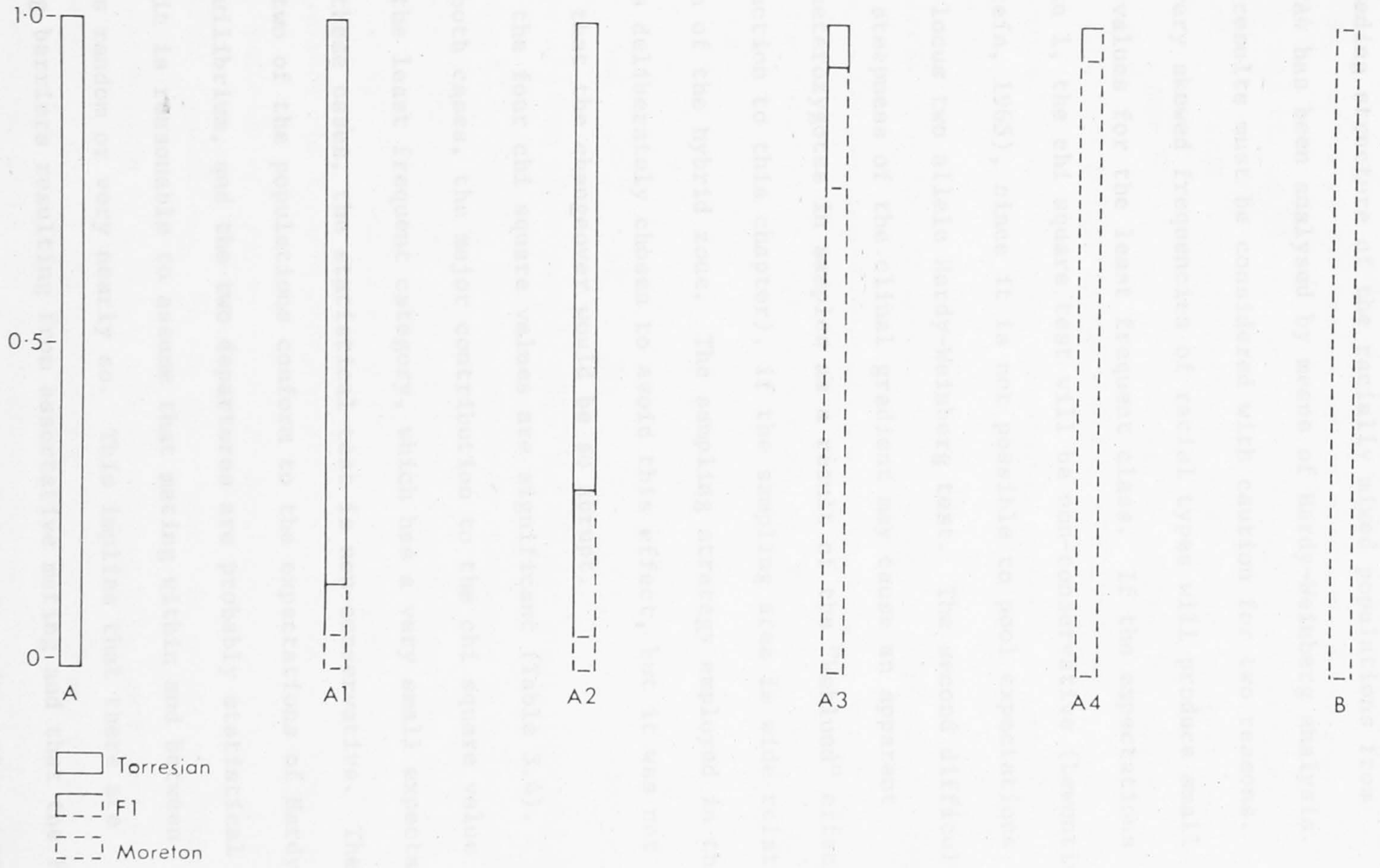
	Males				Females			
Population	No. inv.	S.D.	N	No pure "Torresian"	No. inv.	S.D.	N	No pure "Torresian"
T1PA	1.54	0.69	11	9	1.33	0.49	12	11
T1PA1	1.90	1.10	10	3	2.29	1.73	14	8
T1PA2	2.43	1.51	7	8	1.66	0.82	6	7

TABLE 3.4 χ^2 Analysis of Racial Frequencies

	Racial types					
Pop.	T	F1	M	χ^2	Signif.	Comment
A1	35	3	2	11.14	**	Small cell expectation, <1
A2	29	9	2	1.21	N.S.	
A3	3	8	31	4.13	*	Small cell expectation
A4	0	2	41	0.03	N.S.	

Hardy-Weinberg Analysis of Racial Frequencies in the Contact Zone Populations

Figure 3.4. Racial frequencies in the contact zone. The sampling points in the transect are 200 metres apart.


1·0
0·5
0
A
A1
A2
A3
A4
B
Torresian
F1
Moreton

a steep clinal change (Fig. 3.4, Table 3.4), with the major change in frequency occurring in the 200 metre interval between T1PA2 and T1PA3. T1PA2 contains 72.5% "Torresian" individuals, whereas T1PA3 contains 73.8% "Moreton" individuals. In other words, there is a jump of 65.4% in the frequency of "Torresian" individuals and 68.8% in the frequency of "Moreton" individuals between T1PA2 and T1PA3.

The breeding structure of the racially mixed populations from T1PA1 to T1PA4 has been analysed by means of Hardy-Weinberg analysis. However the results must be considered with caution for two reasons. First, the very skewed frequencies of racial types will produce small expectation values for the least frequent class. If the expectations are less than 1, the chi square test will be non-conservative (Lewontin and Felsenstein, 1965), since it is not possible to pool expectations in a single locus two allele Hardy-Weinberg test. The second difficulty is that the steepness of the clinal gradient may cause an apparent deficit of heterozygotes in samples as a result of the "Wahlund" effect (see Introduction to this chapter), if the sampling area is wide relative to the width of the hybrid zone. The sampling strategy employed in the transect was deliberately chosen to avoid this effect, but it was not anticipated that the changeover would be so abrupt.

Two of the four chi square values are significant (Table 3.4). However in both cases, the major contribution to the chi square value is made by the least frequent category, which has a very small expectation. In both of these cases, the statistical test is non-conservative. Therefore since two of the populations conform to the expectations of Hardy-Weinberg equilibrium, and the two departures are probably statistical artefacts, it is reasonable to assume that mating within and between the races is random or very nearly so. This implies that there are no premating barriers resulting from assortative mating and that the F1 hybrids have normal viability. On the assumption of random mating between the races and normal viability of the F1, the complete change from one race to the other over 1 kilometre, with an abrupt change in the central 200 metre interval, can only be explained in terms of hybrid sterility or hybrid breakdown, which must occur in the subsequent generations.

3. *The Chromosomes in the Contact Zone*

If the frequency of individual chromsomes is followed across the transect, a high level of resolution can be obtained of the genetic changes which are taking place, particularly when consideration is also taken of the racial changes based on total genome analysis. It is possible to compare both the frequencies of a given chromosome between the different points in the transect and also compare the relative frequencies of different chromosomes at the same point (Fig. 3.5, Table 3.5).

As expected from the previous analysis of racial frequencies, the major change in the chromosome frequencies occurs between T1PA2 and T1PA3. In this interval of only 200 metres, the following changes in frequency occur:-

1	2	X	4	5	6	7	8	10	11
.452	.551	.688	.465	.587	.599	.236	.260	.538	.305

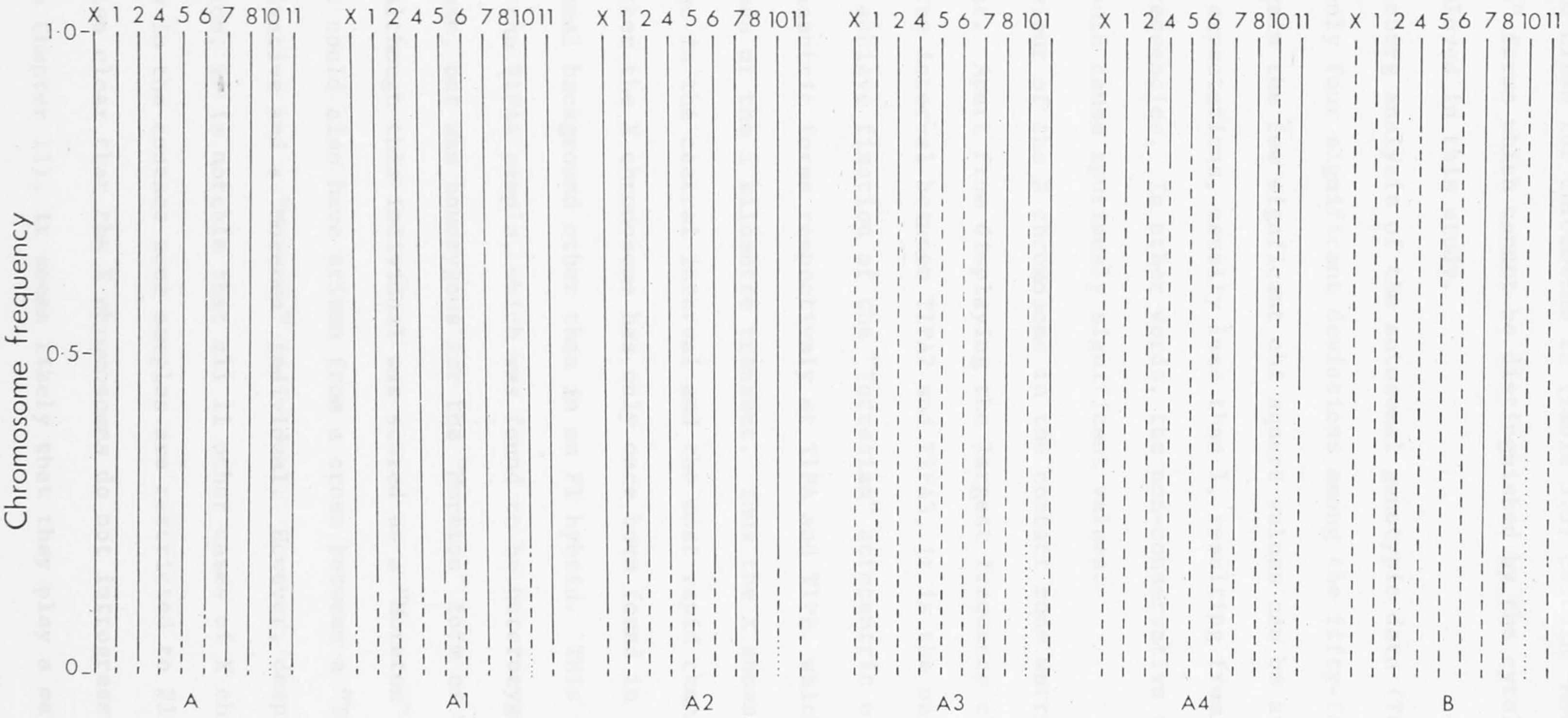
Clearly there are two major groups, with the smaller group, consisting of chromosomes 7, 8, and 11, having frequency changes in this interval of approximately 30% or less. The remaining chromosomes show changes of greater than 45%. It should be noted that the highest change in frequency involves the X chromosome.Further the X chromosome shows the most rapid dimunition of frequencies in the populations on either side of these central populations On the other hand, the smaller frequency changes for chromosomes 7, 8 and 11 are correlated with lower frequencies of submetacentric (7 and 8) and acrocentric (11) chromosomes on the "Moreton: side of the zone. On the basis of the geographical distribution of the forms of these chromosomes (Chapter II), it has previously been concluded that there are primary polymorphisms for these chromosomes,

TABLE 3.5

Population	Sample Size	Chromosome Form	1	2	X	4	5	6	7	8	10	11
T1P3	38	Submeta	0	0	0	0	.013	0	0	0	0	–
		Acro	1.0	1.0	1.0	1.0	.987	–	1.0	1.0	.013	.013
		Telo	–	–	–	–		1.0	–	–	.987	.987
T1Pa	43	Submeta	.035	.023	0	.035	.058	.105	.023	.012	.046	–
		Acro	.965	.977	1.0	.965	.942	–	.977	.988	.035	.012
		Telo	–	–	–	–	–	.895	–	–	.919	.988
T1PA1	40	Submeta	.150	.113	.081	.137	.163	.212	.050	.113	.038	–
		Acro	.850	.887	.919	.863	.837	–	.950	.887	.187	.062
		Telo	–	–	–	–	–	.788	–	–	.775	.938
T1PA2	40	Submeta	.250	.175	.138	.175	.163	.163	.050	.050	.063	–
		Acro	.750	.825	.862	.825	.837	–	.950	.950	.125	.100
		Telo	–	–	–	–	–	.837	–	–	.812	.900
T1PA3	42	Submeta	.702	.726	.826	.640	.750	.762	.286	.310	.226	–
		Acro	.298	.274	.174	.360	.250	–	.714	.690	.500	.405
		Telo	–	–	–	–	–	.238	–	–	.274	.595
T1PA4	43	Submeta	.733	.849	.955	.802	.837	.884	.221	.256	.291	–
		Acro	.267	.151	.045	.198	.163	–	.779	.744	.616	.454
		Telo	–	–	–	–	–	.116	–	–	.093	.546
T1PB	26	Submeta	.885	.865	1.0	.846	.846	.885	.192	.346	.193	–
		Acro	.115	.135	0	.154	.154	–	.808	.654	.692	.615
		Telo	–	–	–	–	–	.115	–	–	.115	.385

The frequencies of chromosomal forms across Transect 1. Note that the acrocentric X in this table is the "Torresian" and not the "Moreton" form of this chromosome. Chromosomes 9 and 12 are omitted from the frequency tabulation since the "Moreton" and "Torresian" forms of these chromosomes cannot be reliably distinguished by the techniques used in this study. The data for T1P4 and T1P5 can be obtained from the frequency tabulation in Chapter 2.

Figure 3.5. Chromosome frequencies in the Contact Zone. Unbroken lines refer to acrocentric-telocentric chromosomes and dashes symbolise submetacentric chromosomes, except for chromosome 10 where dots symbolise submetacentric forms, dashes represent acrocentric forms and unbroken lines refer to the telocentric "Torresian" form of this chromosome.


Chromosome frequency
1·0
0·5
0
X 1 2 4 5 6 7 8 10 11
A
A1
A2
A3
A4
B

similar to the acrocentric-submetacentric polymorphism for chromosome 10. In other words, the acrocentric classes of chromosomes for 7 and 8 and the telocentric class for chromosome 11 (Table 3.5) contain "Moreton" and "Torresian" forms which cannot be distinguished by the cytological techniques employed in this study.

Hardy-Weinberg analysis of the autosomal genotypic data (Table 3.6) has revealed only four significant deviations among the fifty-four tests performed. Again the few signficant chi square values can be attributed to small cell expectations, usually less than 1, resulting from skewed chromosomal frequencies. In other words, the non-conservative nature of the test would cause spuriously significant values.

The behaviour of the X chromosome in the contact zone warrants further comment. Apart from displaying the largest frequency change in the 200 metre interval between T1PA2 and T1PA3, it is the only chromosome to achieve fixation of the "Torresian" acrocentric or the "Moreton" metacentric forms respectively at T1PA and T1PB, which lie at opposite ends of the 1 kilometre transect. Thus the X shows the sharpest change in the central interval and the most rapid change overall. Further the X chromosome has only once been found in a foreign autosomal background other than in an F1 hybrid. This involved a female from the T1PA4 sample, which was found to be heterozygous for the X chromosome, but was homozygous for the "Moreton" form of chromosomes 4, 5 and 6. Although this individual was scored as a "Moreton" backcross derivative, it could also have arisen from a cross between a "Torresian" backcross derivative and a "Moreton" individual. However, despite this single exception, it is notable that all 11 other cases of X chromosome heterozygosity in the contact zone samples are restricted to F1 hybrids. Since it is also clear that the X chromosomes do not introgress in either direction (see Chapter II), it seems likely that they play a major role in maintaining the integrity of the "Torresian" and "Moreton" races. There are several cases where sex chromosome rearrangements are the main

TABLE 3.6 Hardy-Weinberg Analysis of Chromosomal Genotypic Data.

	Chromosome								
Population	1	2	4	5	6	7	8	10	11
T1PA	0.08 N.S.	0.03 N.S.	0.08 N.S.	0.15 N.S.	0.58 N.S.	0.03 N.S.	0.01 N.S.	0.34(1) N.S.	0.01 N.S.
T1PA1	6.78 **	5.55 *	2.77 N.S.	1.20 N.S.	0.58 N.S.	0.11 N.S.	0.61 N.S.	1.39(1) N.S.	0.18 N.S.
T1PA2	0.18 N.S.	0.72 N.S.	0.06 N.S.	0.004 N.S.	0.004 N.S.	0.11 N.S.	8.97 ***	0.42(1) N.S.	1.11 N.S.
T1PA3	0.04 N.S.	2.06 N.S.	0.53 N.S.	0.10 N.S.	0.28 N.S.	0.19 N.S.	0.55 N.S.	5.18(3) N.S.	0.006 N.S.
T1PA4	0.04 N.S.	5.76 *	0.29 N.S.	0.29 N.S.	0.75 N.S.	0.008 N.S.	0.42 N.S.	5.24(3) N.S.	0.27 N.S.
T1PB	0.44 N.S.	0.63 N.S.	0.34 N.S.	0.34 N.S.	1.59 N.S.	1.48 N.S.	0.92 N.S.	1.31(2) N.S.	3.18 N.S.

* 5% ** 1% N.S. Not significant

In both cases of highly significant X^2 values, cell expectations of less than 1 for the least frequent class contribute disproportionately to the value. For chromosome 8 of T1PA2, 8.10 of a total X^2 value of 8.97 is contributed in this way. A three allele test has been applied to the chromosome 10 data, and the degrees of freedom are shown in brackets after the chi square value.

or, indeed, the sole means of distinguishing between parapatrically distributed taxa, which meet in a narrow zone of hybridization, and are therefore assumed to play a role in maintaining the isolation of these taxa. Examples are found in the *Didymuria violescens* complex (Craddock, 1971), the Viatica group of Morabine grasshoppers (White *et al.*, 1967; Mrongovius, 1975) and the Alpine grasshopper, *Podisma pedestris* (Hewitt, 1975). The major rearrangement difference in each case is an X-autosome fusion and the F1 hybrids males consequently cannot suffer from infertility ensuing from the structural change. For example, only a 3% increase in the frequency of asynapsis has been demonstrated for male hybrids of P(24XY) and viatica (17) (Mrongovius, 1975), and the fecundity of these hybrids was, in general, quite normal. All of these cases, including that of the "Moreton" and "Torresian" races of *Caledia*, imply that the gene content of the sex chromosomes may be acting as an important isolating mechanism between the taxa, presumably by epistatic interactions with the autosomal genes.

4. *Gametic Disequilibrium Between Chromosomes*

For two loci, each with two alleles, there are nine genotypic categories. Therefore, an adequate statistical analysis of non-random associations between two loci (or non-homologous chromosomes) requires large sample sizes, particularly when the gene frequencies are very skewed. In this case, the analysis of non-random associations between non-homolgous chromosomes in the populations sampled from the hybrid zone must overcome both the problem of skewed chromosome frequencies and relatively small sample sizes.

The statistical analysis used here consists of a "genic" independence chi square test, which has been applied to all possible pairwise combinations of chromosomes 1, 2, 4, 5, 6, 10 and 11 for populations T1PA1, T1PA2, T1PA3 and T1PA4. The acrocentric and metacentric forms of chromosome 10 have been pooled for this analysis. All cells with an expectation of less than one in the independence test matrix have been pooled with the adjacent cell with the smallest expectation. The process has been continued until all expectations less than one have been pooled

TABLE 3.7

T1PA2 (2-5) 2

		I/I	I/B	B/B	
I/I	O	1	0	0	1
	E	.032	.307	.723	
	X^2				
5 I/B	O	0	7	4	11
	E	.334	3.152	7.429	
	X^2		4.699	1.582	
B/B	O	1	3	24	28
	E	.858	8.092	19.073	
	X^2		3.204	1.273	
		2	10	28	40

Pooled cells Observed : 2

Expected : 2.254

Component of X^2 : .028

Total X^2 = 10.786

Degrees of freedom = 2

Example of chromosomal independence analysis for chromosomes 2 and 5 and the pooling strategy used throughout this analysis.

and the total pooled expectation is greater than one. A maximum of 5 cells can be pooled since one degree of freedom is lost for each pooled category. This strategy ensures that the test is statistically conservative (Lewontin and Felsenstein, 1965), although information is lost by combining the observations from different genotypic categories. An example of the analysis is presented in Table 3.7. In this case, the parental double homozygous and the double heterozygous classes are in excess of expectation. The "recombinant" classes, on the other hand, are observed less frequently than expected. All cases of statistically significant departures from non-randomness are due to associations of this type, with an excess of parental combinations in the double homozygous and double heterozygous categories. However, the contribution of the less frequent parental double homozygous type is inevitably lost because of the necessity of combining small cell expectations.

There is a striking difference in the pattern of gametic disequilibrium between non-homologous chromosomes in populations on either side of the contact zone (Table 3.8). Thus T1PA1 and T1PA2, which are predominantly "Torresian", have strong non-random associations, which affect all of the tested chromosomes. Although not all pairwise combinations show statistically significant disequilibria, all display the same trend with an excess of parental double homozygous and double heterozygous types. However, there is no consistent pattern of association in T1PA3 and T1PA4, which are predominantly "Moreton" in constitution, except for the chromosome 1-2 combination, which shows statistically significant departures from non-randomness in both populations. The other departures in these populations are few and only marginally significant, compared with the many highly significant associations in T1PA1 and T1PA2. The change from populations with many significant non-random associations to those with few detectable disequilibria occurs between T1PA2 and T1PA3. This corresponds to

TABLE 3.8

CHROMOSOMES

T1PA1	2	4	5	6	10	11
1	14.29(2)	8.85(2)	12.10(2)	7.31(3)	11.95(3)	6.46(2)
	***	*	***	N.S.	**	*
2		6.64(2)	11.97(2)	11.03(3)	8.99(3)	13.48(2)
		*	***	*	*	***
4			7.29(2)	3.89(3)	2.79(3)	9.17(2)
			*	N.S.	N.S.	**
5				3.79(3)	7.56(3)	1.73(2)
				N.S.	N.S.	N.S.
6					7.21(4)	7.45(2)
					N.S.	*
10						5.63(3)
						N.S.

T1PA2	2	4	5	6	10	11
1	16.42(4)	12.30(3)	11.62(3)	6.62(3)	4.96(3)	3.61(2)
	***	**	**	N.S.	N.S.	N.S.
2		23.06(2)	10.79(2)	12.41(2)	5.85(2)	3.15(2)
		***	***	***	N.S.	N.S.
4			14.60(2)	16.50(2)	8.45(2)	6.27(2)
			***	***	*	*
5				16.15(2)	5.03(2)	9.80(2)
				***	N.S.	**
6					6.22(2)	9.80(2)
					*	**
10						2.88(2)
						N.S.

TABLE 3.8 (contd)

T1PA3	2	4	5	6	10	11
1	12.08(5) *	6.80(5) N.S.	7.35(5) N.S.	8.81(5) N.S.	4.74(5) N.S.	9.72(5) N.S.
2		1.82(5) N.S.	4.77(5) N.S.	13.44(5) *	8.02(5) N.S.	10.29(5) N.S.
4			4.02(5) N.S.	6.01(5) N.S.	5.82(5) N.S.	4.88(5) N.S.
5				13.88(5) *	5.89(5) N.S.	7.37(5) N.S.
6					11.76(5) *	5.98(5) N.S.
10						4.82(5) N.S.

T1PA4	2	4	5	6	10	11
1	9.79(3) *	0.65(3) N.S.	2.79(3) N.S.	1.06(3) N.S.	3.14(3) N.S.	1.88(5) N.S.
2		3.28(3) N.S.	5.06(2) N.S.	3.11(2) N.S.	4.14(2) N.S.	1.54(3) N.S.
4			2.38(3) N.S.	6.11(2) *	0.77(2) N.S.	3.00(4) N.S.
5				1.53(2) N.S.	1.01(2) N.S.	4.44(4) N.S.
6					2.58(2) N.S.	0.95(3) N.S.
10						4.28(3) N.S.

* $P < .05$ Degrees of freedom in parentheses

** $P < .01$

*** $P < .005$

N.S. Not significant

Gametic disequilibrium analysis for pairwise combinations of chromosomes in populations from Transect 1. The analysis has been performed as shown in Table 3.7.

the previously described change from a predominantly "Torresian" population to one which is mainly "Moreton" in constitution, with a simultaneous large change in the frequencies of all chromosomes over this 200 metre interval.

The "recombinant" chromosomal genotypes consist of those pair-wise categories in which one chromosome is heterozygous and the other homozygous (e.g. I/B, B/B) and those in which both are homozygous for a combination not found in the parental types (e.g. I/I, B/B) (see Table 3.7). These genotypes will be produced either by backcrossing or the crossing *inter se* by the F1 hybrids. The deficit in the recombinant categories in the predominantly "Torresian" populations suggests that there is a severe restriction on the production of F2 and backcross progeny in these populations, although the data clearly show that it is not an absolute restriction. On the other hand, the general lack of associations between autosomal forms on the "Moreton" side of the contact zone demonstrates that there is much less restriction to the formation of new autosomal combinations in this case. However in both types of populations, with one possible exception, all females heterozygous for the X chromosome are F1 hybrids. Furthermore, the homozygous X chromosome pair in the females has been found to be diagnostic of their racial type in the hybrid zone populations. In other words, homozygous metacentric X individuals are "Moreton" and the homozygous acrocentric X females are always otherwise classifiable as "Torresian". The X-autosome associations in the females have not been tested because of the reduction in sample size when the male data are removed. Nevertheless, it is clear that there are barriers to the formation of new X chromosome-autosome combinations.

Severe selection is required to maintain associations between even a single pair of non-homologous chromosomes in the face of independent segregation. Further the recombination load increases multiplicatively for each additional chromosome pair. The genetic load can be minimized, although it will still be severe, if selection acts early and simultaneously eliminates several new chromosomal combinations. Hybrid breakdown is one possible means of restricting the formation of new chromosomal combinations on the "Torresian" side of the zone and is the only mechanism likely to maintain so many non-random associations, without a very severe genetic load. However the lack of non-random associations on the "Moreton" side of the zone implies that backcross breakdown is less severe and would permit selective autosomal introgression. If the differences in the pattern of chromosomal associations arise in this way, backcross breakdown must be more severe in crosses to the "Torresian" parents than in crosses to the "Moreton" parental types.

The results of the gametic disequilibrium analysis on these four populations at the contact zone are consistent with the pattern of one way introgression of chromosomes from the "Torresian" into the "Moreton" race. Furthermore, asymmetrical hybrid breakdown would not only give rise to the observed pattern of disequilibria and the restrictions on introgression, but would also maintain a narrow hybrid zone. Although there is evidence from a laboratory hybridization experiment of severe hybrid breakdown (see Chapter IV), the mechanism of unilateral backcross breakdown has not been elucidated.

Summary of Results

In the 200 metre interval between T1PA2 and T1PA3, there are highly significant increases in the average number of inversions per individual of 7.06 for the males and 10.61 for the females. Furthermore, in populations to the west of T1PA2 there is a very rapid elimination of "Moreton" chromosomes from the "Torresian" background. On the other hand, there appears to be either incorporation or at least a much slower loss of "Torresian" chromosomes from the "Moreton" side of the contact zone.

The change in the number of inversions per individual between T1PA2 and T1PA3 corresponds to a change from a predominantly "Torresian" population to a predominantly "Moreton" one. In this interval, there is a 65% decrease in the frequency of "Torresian" individuals and a 69% increase in the frequency of "Moreton" individuals, although this distance is well within the range of dispersal of an adult *Caledia*. Sympatry between the races is restricted to approximately 1 kilometre, although for at least 800 metres of this distance, the less common "foreign" racial type is found at a frequency below 5-7%. Consequently, even though the analysis of the racial and hybrid frequencies indicated random mating and normal viability of the F1 hybrids (see also Chapter IV), the production of hybrids will be restricted by this geographical distribution of chromosomal types.

The pattern of change of the chromosomes is similar to the change in racial frequencies. However the X chromosome shows the most extreme behaviour with the largest frequency change between T1PA2 and T1PA3. It is also the only chromosome to achieve fixation for the acrocentric morph at T1PA and the submetacentric morph at T1PB at the opposite ends of the one kilometre transect. The autosomes show a general compliance with the Hardy-Weinberg equilibrium.

The chromosomal independence test has revealed differences in the pattern of association between autosomes on the "Torresian" and "Moreton" sides of the contact zone. In T1PA1 and T1PA2, significant interchromosomal associations are found in 13-14 of the 21 tested combinations (Table 3.8), with an excess of the parental combinations of chromosomal morphs in each case. In T1PA3 and T1PA4 on the "Moreton" side of the zone, there are only 2-4 marginally significant non-random associations. The maintenance of the parental autosomal combinations in T1PA1 and T1PA2 can be explained by severe hybrid breakdown, which would also explain the prevention of introgression of "Moreton" chromosomes into the "Torresian" race.

The similarities and differences between these populations on either side of the contact zone are summarized in Table 3.9. In both

TABLE 3.9

		"Torresian" side	"Moreton" side
Similar	i)	Rapid elimination of "Moreton" metacentric X chromosome	Rapid elimination of "Torresian" acrocentric X chromosome
	ii)	All autosomes in Hardy-Weinberg equilibrium	All autosome in Hardy-Weinberg equilibrium
	iii)	Random mating between the races	Random mating between the races
Different	i)	Rapid elimination of foreign autosomes	Retention of foreign autosomes -introgression
	ii)	Many, strong non-random associations between the autosomes	Few, weak non-random associations between the autosomes
	iii)	Severe backcross breakdown	Less severe breakdown

Summary of the similarities and differences between the "Torresian" (up to T1PA2) and "Moreton" (from T1PA3) sides of the contact zone.

races, the X chromosome appears to play an important isolating role. In the females, the X chromosome constitution is diagnostic of racial or F1 hybrid status. Further, the X chromosome never introgresses and has the steepest changeover gradient in the contact zone. Autosomal interactions also appear to be important in preventing the introgression of "Moreton" chromosomes into the "Torresian" race.

DISCUSSION

1) The "Moreton" X chromosomes and introgression

The nature of the geographical distribution of the acrocentric and metacentric morphs of the "Moreton" X chromosomes (see Chapter II) permits a comparison of the rates of introgression of "Torresian" autosomes into "Moreton" populations fixed for either of these forms of the X chromosome. Although a detailed transect has been made only between the "Torresian" and "Moreton" metacentric X populations, there are still obvious differences between the "Moreton" acrocentric X and metacentric X populations from the vicinity of the contact zone. The comparisons can be best made between the Spring Valley Creek population (see Chapter II), which is fixed for the acrocentric X and T1PB (Figure 3.5, Table 3.5), which are in approximately equivalent positions relative to the contact zone. The introgressed acrocentric-telocentric morphs of chromosomes 1, 2, 10 and 11 are found at a much higher frequency in the Spring Valley Creek population than in T1PB. Figure 3.6 shows a side by side comparison of the frequencies between these populations and clearly reveals the differences. However the presumed introgressed morphs of chromosomes 4, 5, 6, 7 and 8 have similar frequencies between the two types of populations. This difference between acrocentric X and metacentric X populations is consistently observed in other "Moreton" populations collected in the vicinity of the contact zone. For example, it can be seen, in a comparison of acrocentric X populations such as Walliebum Waterhole (5), Maryborough (6), Tiaro (7), Redbank (8) and Rosemount (9) with those carrying a metacentric X such as Kenilworth (18),

Figure 3.6 Comparison of Chromosomal Frequencies at Spring Valley Creek (a) and T1PB (b). Dots refer to the "Moreton" acrocentric X chromosome (a) and the metacentric form of chromosome 10 (b). Dashes symbolise submetacentric chromosomes and the unbroken lines refer to acrocentric-telocentric "Torresian" chromosomes.

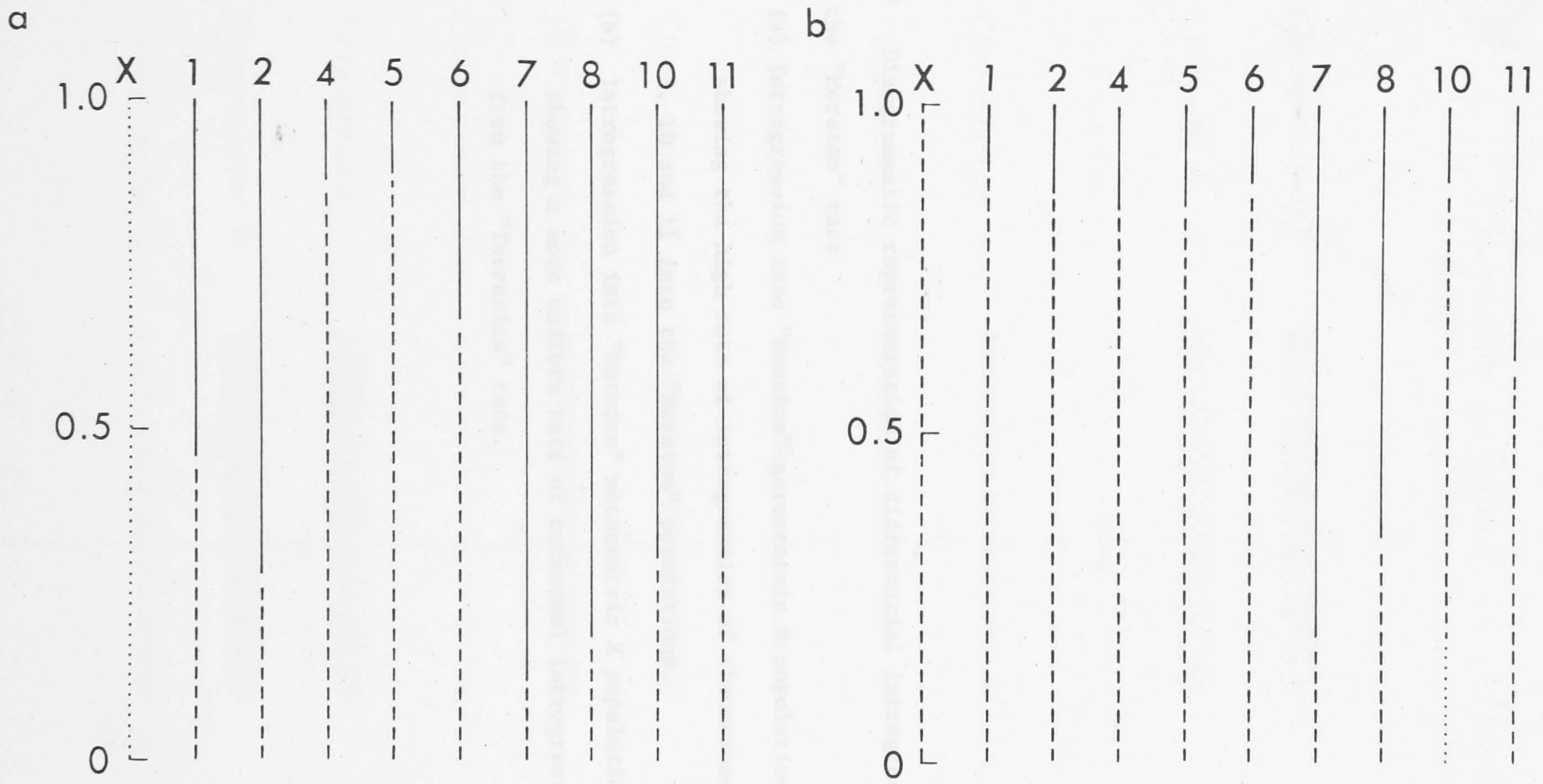
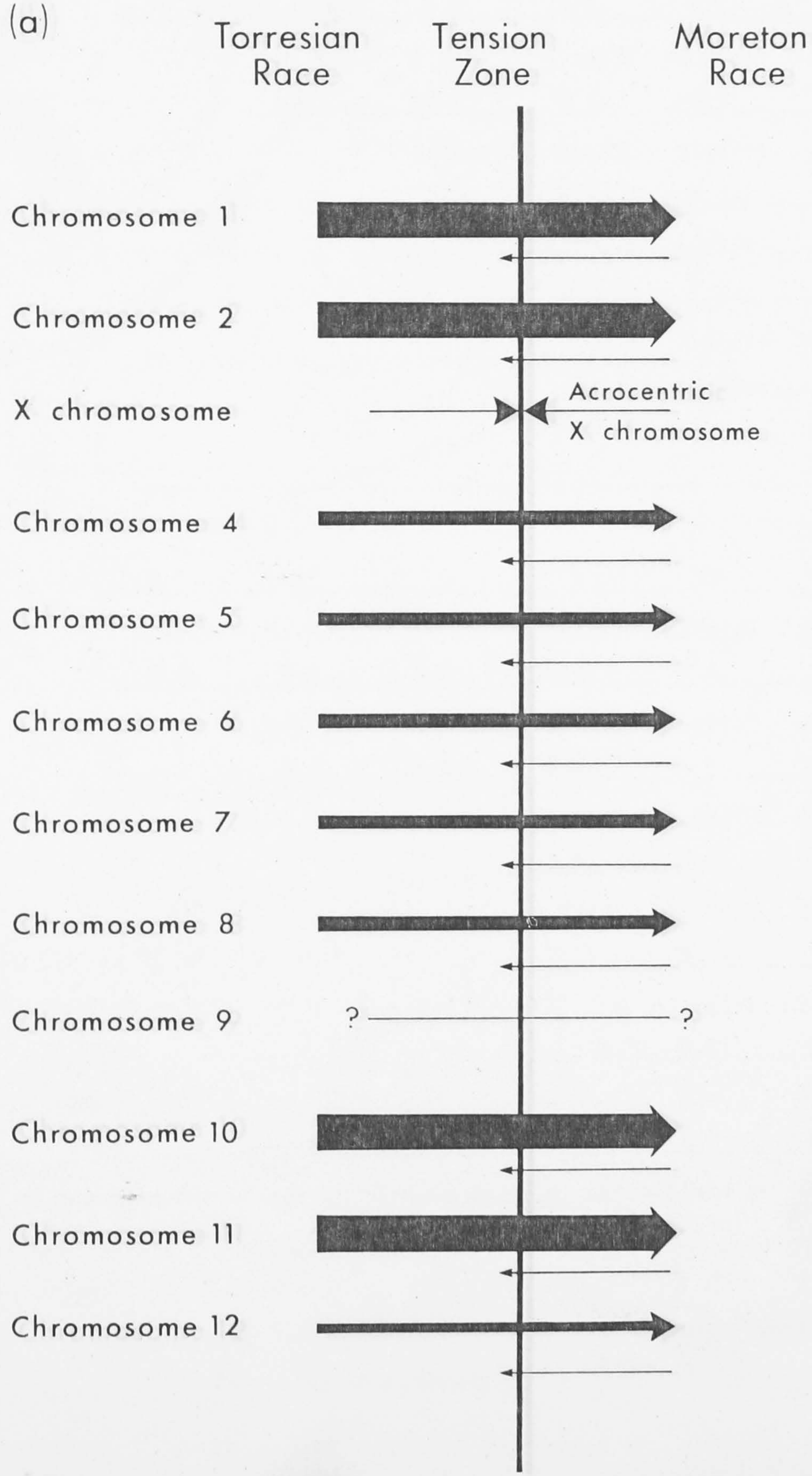

a
X
1
2
4
5
6
7
8
10
11
1.0
0.5
0
b
X
1
2
4
5
6
7
8
10
11
1.0
0.5
0

Figure 3.7 Diamgrammatic representation of differential introgression into the "Moreton" race

(a) Introgression into "Moreton" acrocentric X populations, showing the high rate of introgression of chromosomes 1, 2, 10 and 11 into the "Moreton" populations.

(b) Introgression into "Moreton" metacentric X populations showing a more uniform rate of autosomal introgression from the "Torresian" race.


(a)
Torresian Race
Tension Zone
Moreton Race
Chromosome 1
Chromosome 2
X chromosome
Acrocentric X chromosome
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
?
?
Chromosome 10
Chromosome 11
Chromosome 12

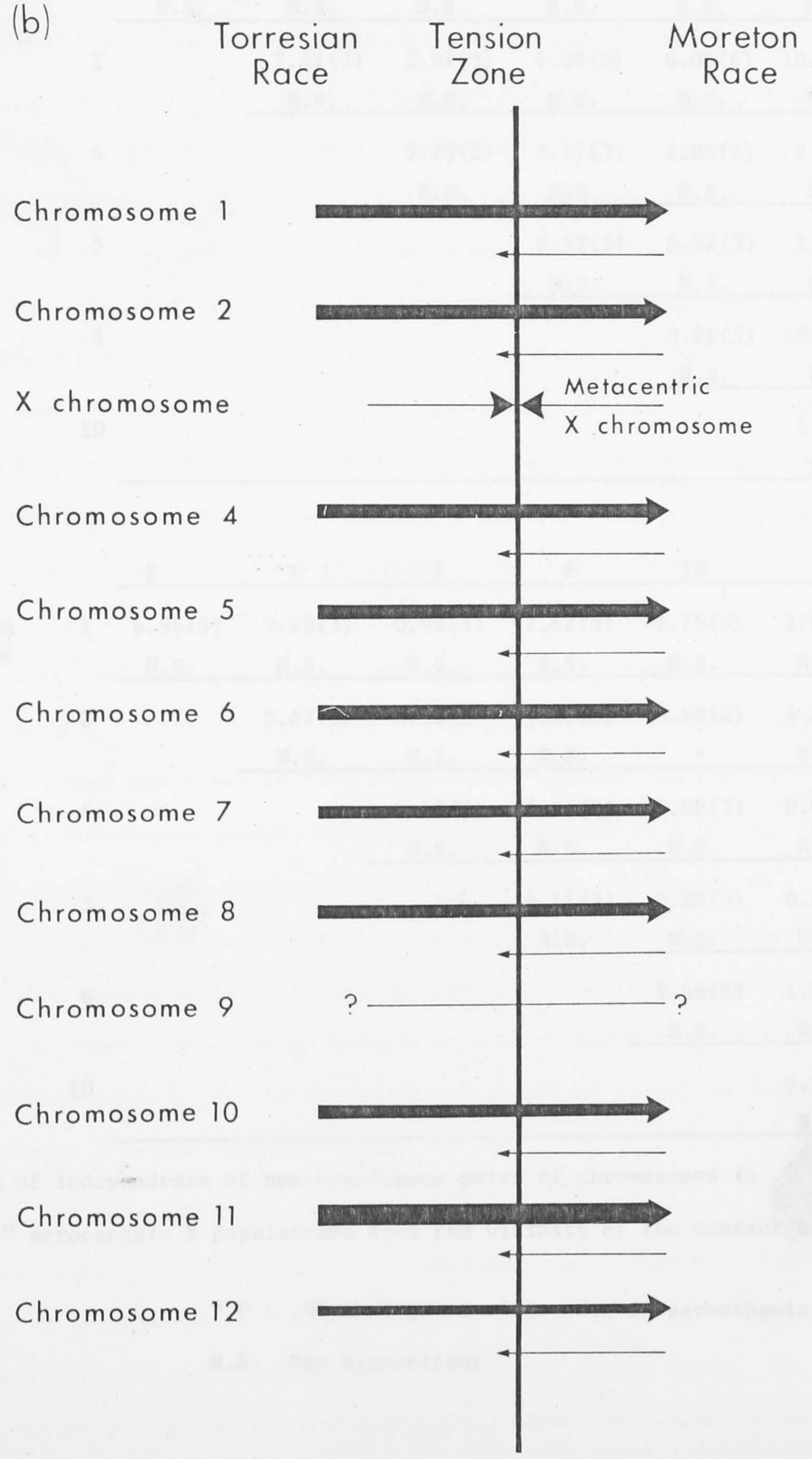

(b)
Torresian Race
Tension Zone
Moreton Race
Chromosome 1
Chromosome 2
X chromosome
Metacentric X chromosome
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
?
?
Chromosome 10
Chromosome 11
Chromosome 12

TABLE 3.10

		2	4	5	6	10	11
Spring Valley Creek	1	4.93(5) N.S.	2.34(3) N.S.	2.51(3) N.S.	5.34(6) N.S.	6.43(6) N.S.	2.52(6) N.S.
	2		7.41(3) N.S.	2.97(3) N.S.	4.09(5) N.S.	6.06(6) N.S.	10.36(5) N.S.
	4			5.29(2) N.S.	3.17(3) N.S.	2.08(3) N.S.	2.69(3) N.S.
	5				2.52(3) N.S.	6.82(3) N.S.	1.88(3) N.S.
	6					3.21(5) N.S.	10.03(5) N.S.
	10						2.91(5) N.S.

		2	4	5	6	10	11
Walliebum Waterhole	1	6.96(5) N.S.	2.28(3) N.S.	0.99(3) N.S.	2.82(5) N.S.	2.75(5) N.S.	2.51(4) N.S.
	2		5.47(3) N.S.	6.81(3) N.S.	9.36(5) N.S.	9.59(4) *	4.89(4) N.S.
	4			2.48(2) N.S.	5.66(3) N.S.	4.08(3) N.S.	0.68(2) N.S.
	5				4.11(3) N.S.	7.29(3) N.S.	0.42(3) N.S.
	6					5.46(5) N.S.	1.18(4) N.S.
	10						6.37(4) N.S.

Analysis of independence of non-homologous pairs of chromosomes in "Moreton" acrocentric X populations from the vicinity of the contact zone.

* $P < .05$ Degrees of freedom in parenthesis

N.S. Not significant

Scrubby Creek (19), Villeneuve (21) and Mt. Archer (22) (see Chapter II for chromosomal frequencies and location of these populations). In these general comparisons, account must be taken of the distance of the populations from the contact zone, since there is a general dimunition in the frequency of the introgressed chromosomes with greater distance from the contact zone. A simplified diagrammatic representation of this differential introgression has been made in Figure 3.7. The large arrows for chromosomes 1, 2, and 11 (Fig. 3.7a) represent a higher frequency of introgression of these chromosomes into the acrocentric X populations both relative to the other autosomes in these populations and to the similar autosomes in the metacentric X populations.

Because of the differences in the frequencies of these chromosomes between the acrocentric X and metacentric X populations, the independence of non-homologous chromosomal morphs has been tested in the Spring Valley Creek and Walliebum Waterhole populations to determine whether they differ in their pattern of associations from T1PA2 and T1PA3 (Table 3.8). Both of these acrocentric X populations have been collected very close to the contact zone and in the Walliebum Waterhole population, a "Torresian" backcross female was taken in the predominatly "Moreton" sample. The analysis of non-random associations in these cases (Table 3.10) agrees with the results in the populations from Transect 1. There are no significant pairwise associations in the Spring Valley Creek population and only one marginally significant association in the Walliebum Waterhole sample. Epistatic interactions between autosomes, which tend to maintain parental combinations, are therefore not an important factor in regulating introgression into the "Moreton" race.

2) A model of hybrid breakdown in a contact zone

A deterministic model of hybrid breakdown in a contact zone has been produced to analyse the genetic load and the change in racial frequency per generation with any given initial frequency of racial types. The model (see Table 3.11) assumes random mating between the races, normal

TABLE 3.11. A deterministic model of the effects of hybrid breakdown in a contact zone. The model allows the calculation of the genetic load and expected change in racial frequencies at any given racial frequency. The model assumes that mating between the races and their derivatives is at random and determines the consequences of breakdown in the second generation after the races make contact. It is possible to extrapolate to subsequent generations.

	TT		MM
	p		q

↓

	TT	TM(F1)	MM
Gen 1	p^2	$2pq$	q^2

Gen 2

TT	T Backcross	F2	TM(F1)	M Backcross	MM
p^4	$4p^3q$	$4p^2q^2$	$2p^2q^2$	$4pq^3$	q^4
1	$1-s_1$	$1-s_2$	1	$1-s_3$	1

$$\text{Load} = 4p^3q\,s_1 + 4p^2q^2s_2 + 4pq^3s_3$$

$$\hat{q} = \frac{q^4 + 4pq^3(1-s_3) + p^2q^2 + \frac{4}{3}\,p^2q^2(1-s_2)}{1 - 4p^3q\,s_1 - 4p^2q^2\,s_2 - 4pq^3\,s_3}$$

$$\Delta q = \hat{q} - q$$

Regimes of Hybrid Breakdown (see Fig. 3.8a,b)

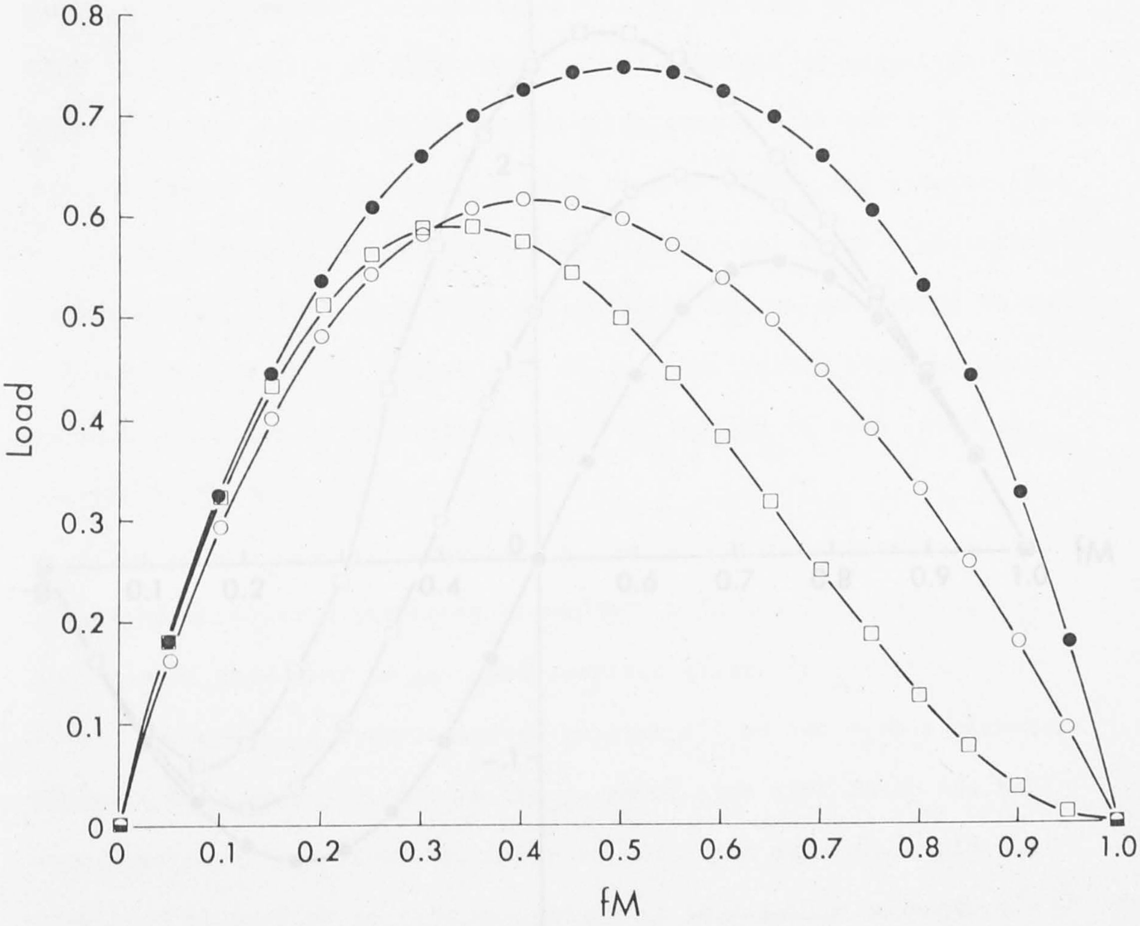
	s_1	s_2	s_3	Symbol
1	1.0	1.0	1.0	●
2	1.0	1.0	0	□
3	0.9	1.0	0.5	○

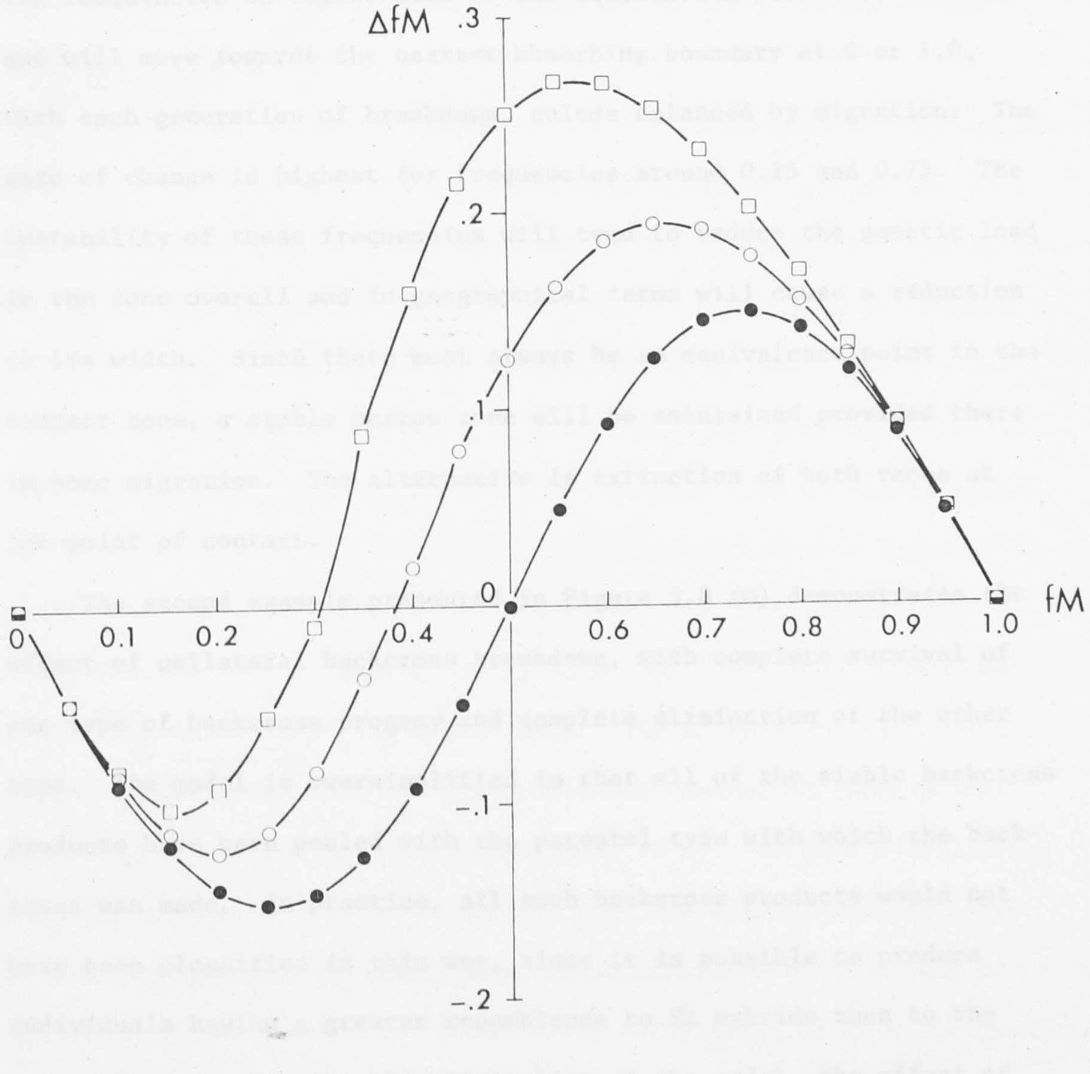
Figure 3.8 Effects of hybrid breakdown in a hybrid zone.

(a) Genetic load at any given racial frequency in the zone.

(b) The expected change in racial frequency at any given initial frequency after one generation of hybrid breakdown.

Three different regimes of hybrid breakdown are presented according to Table 3.11.


Load
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
fM



ΔfM
.3
.2
.1
0
−.1
−.2
0
0.1
0.2
0.4
0.6
0.7
0.8
0.9
1.0
fM

viability and fertility of the F1 hybrids and several different regimes of F2 and backcross breakdown.

With complete F2 and backcross breakdown, there is a symmetrical relationship for both genetic load and the change in racial frequency (Fig. 3.8a,b●) about the equivalence point of the contact zone, where both races occur with equal frequency and make an equal contribution to the derivatives. At this mid-point, the maximum genetic load of 75% occurs and the expected change in racial frequency is 0. However the frequencies on either side of the equilibrium point are unstable and will move towards the nearest absorbing boundary at 0 or 1.0, with each generation of breakdown, unless balanced by migration. The rate of change is highest for frequencies around 0.25 and 0.75. The instability of these frequencies will tend to reduce the genetic load in the zone overall and in geographical terms will cause a reduction in its width. Since there must always be an equivalence point in the contact zone, a stable narrow zone will be maintained provided there is some migration. The alternative is extinction of both races at the point of contact.

The second example presented in Figure 3.8 (□) demonstrates the effect of unilateral backcross breakdown, with complete survival of one type of backcross progeny and complete elimination of the other type. The model is oversimplified in that all of the viable backcross products have been pooled with the parental type with which the backcross was made. In practice, all such backcross products would not have been classified in this way, since it is possible to produce individuals having a greater resemblance to F1 hybrids than to the parental type. Despite this limitation of the model, the effect of asymmetrical breakdown is to displace the equilibrium frequency and point of maximum genetic load away from 0.5. Again the equilibrium is unstable and frequencies on either side of the null point will move towards the absorbing boundaries.

The results of 90% breakdown in one of the backcrosses and 50% breakdown in the other are in general intermediate to the two previously described examples (Fig. 3.8a,bO). The equilibrium frequency and point of maximum genetic load occur at approximately 0.4. The frequencies on either side of the equilibrium point will again move towards one of the absorbing frequency boundaries at 0 or 1.0. In both of the latter two examples, there will be an apparent deficit of hybrids because of the inclusion of the surviving backcross progeny in the parental classes. However this effect is reduced in relation to the severity of the backcross breakdown.

Theoretical models of narrow clines have demonstrated that asymmetrical gene flow will move the cline without appreciably affecting its other characteristics such as width (Endler, 1977). Asymmetrical hybrid breakdown will also cause the position of a narrow cline to move because of the far greater survival on the side of the cline which suffers least from hybrid breakdown. However in this case, the cline will move in the opposite direction to the direction of gene flow or introgression. If the model can be validly applied to the hybrid zone in *Caledia*, it would mean that the "Moreton" race has advanced, and may still be advancing, on the "Torresian" race, acquiring "Torresian" chromosomes during the movement of the hybrid zone. The movement of the hybrid zone will continue until an environmentally determined geographical equilibrium position is established (Key, 1974). At this point, there will be an increase in the fitness of the "Torresian" race, relative to the introgressed "Moreton" race, to counterbalance the effect of the asymmetrical breakdown. However, in the absence of an environmentally determined fitness gradient, the hybrid zone will continue to move unimpeded.

Although migration of the parental racial types is necessary for the maintenance of a hybrid zone, it has not been considered specifically in this model. However, if a zone of overlap is formed and there is no subsequent migration, the less frequent "foreign" racial type will become extinct on both sides of the zone, except at the equilibrium point, where there will be extinction of both racial types. Both the differences in population density on either side of the zone and the fact that the double extinction point is not at a frequency of 0.5, explain the tendency for the position of the hybrid zone to move under a regime of asymmetrical hybrid breakdown. The rate of migration and tendency for dispersal are not known for these races of *Caledia*. Therefore the relationship between migration and rate of dispersal cannot be considered in detail. However the extreme narrowness of the contact zone with the bulk of the transition occurring in a 200 metre interval, makes it likely that hybrid breakdown is severe and that migration occurs at a low rate for distances of approximately one kilometre.

Severe F2 breakdown has already been demonstrated in laboratory hybridization of the "Moreton" and "Torresian" races. Further, the asymmetry in the pattern of chromosomal disequilibria in the contact zone populations provides evidence for the differential effect of hybrid breakdown on the two races. The model of hybrid breakdown may have many applications in the study of narrow contact zones since it can explain both the presence of a stable narrow zone and also selective introgression.

CHAPTER IV

Experimental Hybridization of the "Moreton" and "Torresian" Races

INTRODUCTION

Experimental hybridisation provides the only adequate test of the level of incompatibility which has been achieved between the differently coadapted gene pools of closely related species. When the analysis of fertility includes both progeny production and meiotic analysis of the hybrids, it permits the assessment of the genetic divergence relevant to the maintenance of the integrity of the given taxa. Other estimates of intertaxon divergence, such as genetic distance statistics based on electrophoretic data (Ayala, 1975), while useful, do not bear any necessary relevance to those factors which are important in preventing the amalgamation of the gene pools.

The frequency with which hybridization experiments have been performed is in general a function of the ease with which the species can be bred, maintained and crossed under laboratory conditions. Consequently the genus *Drosophila* is well represented in the literature on hybridization studies because of the precision, speed and ease with which such experiments can be performed in the laboratory. An example of the complete spectrum of postmating reproductive isolating mechanisms that can be found within this genus is seen in the Hawaiian *Drosophila* (Craddock, 1974a). Here they range from F1 hybrid inviability during the early stages of development [i.e. in crosses between *D. balioptera* and *D. villosipedis* (Yang and Wheeler, 1969)] to complete fertility of F1 and F2, with segregation of species specific morphological characteristics in the F2 and subsequent generations [i.e. in hybridizations between *D. silvestris* and *D. heteroreura* (Ahearn and Val, 1975; Craddock, 1974b)]. However behavioural isolation and insemination reactions are important factors in preventing hybridization in the

Hawaiian *Drosophila* (Craddock, 1974a). In particular, *D. silvestris* and *D. heteroneura* show strong behavioural isolation. Levels of postmating isolation intermediate to the examples already quoted can be found in other Hawaiian species (Craddock, 1974a) and also in species of the *willistoni* group of *Drosophila* (Ayala *et al.*, 1974). In the latter group for example, in crosses between *D. willistoni willistoni* and *D. w. quechua*, the male hybrids are sterile from one of the reciprocal crosses, but are not sterile when derived from the cross in the other direction. On the other hand, *D. equinoxalis equinoxalis* and *D. e. carribensis* produce sterile male hybrids but fertile females independently of the direction of the cross.

F2 and backcross breakdown are often found in *Drosophila* hybrids, even when the viability and fitness of the F1 hybrids equals or exceeds that of the parental types (Endler, 1977). This has been particularly well analysed for *D. pseudoobscura* and *D. persimilis*, where as for the subspecies of *D. equinoxalis*, the male F1 hybrids are sterile but the female hybrids are not. In backcrosses of the F1 females to either of the two parental species, the viability of the progeny is low, when compared to the parental species and the F1 (Dobzhanky, 1970). Experimental studies by Dobzhansky, using markers on all chromosomes, have demonstrated that the breakdown is caused by genic factors on all chromosomes.

Among the vertebrates, species of Anura have been studied most frequently by experimental hybridization. Behavioural barriers to interbreeding, which are very important in frogs, can be overcome by an artificial fertilization procedure (Moore, 1957). Developmental compatibility of the species can be analysed in detail because of the large size of the eggs and developing embryos (Mecham, 1960, 1961; Straughn and Main, 1968; Cuellar, 1971; Bull, 1975). A spectrum of hybrid compatibilities has been encountered which shows a distinct similarity to the situation in *Drosophila*, although many of the reported cases are concerned with F1 inviability only. In other groups of vertebrates, hybridization studies are infrequent and usually have not involved a quantitative analysis of progeny production. In the Urodeles for example, hybridization of races and species of *Triturus* (White, 1946; Callan and Spurway, 1951) and *Taricha* (Twitty, 1964) has been performed and F1 hybrids produced. Although the *Triturus* hybrids, in particular the males, are generally sterile, hybrids between salamander species of the genus *Taricha* show little or no reduction of fertility in either sex. In the mammals, hybrids have been produced between chromosomally distinct taxa. For example, they have been produced between *Mus musculus*, with 2n = 40, and two different fusion races, both with 2n = 22. There is a drastic reduction in fertility in all hybrids because of the malorientation of the resulting large multivalents (Capanna *et al.*, 1976). On the other hand, hybrids between *Equus caballus*, with 2n = 64, and *Equus przewalskii*, with 2n = 66, are fully fertile (Benirschke, Maloef, Low and Heck, 1965).

Interspecific hybrids of grasshoppers, both natural and artificially produced, have been examined in several cases. The emphasis has usually been directed toward an analysis of hybrid meiosis (Klingstedt, 1939; Carothers, 1941; Helwig, 1955; White, 1957; John and Lewis, 1965; Mrongovius, 1975). Again, there is a spectrum of fertility in the hybrids. The F1 hybrids between *Eyprepocnemis plorans plorans* and *E. p. ornatipes* are almost completely sterile (John and Lewis, 1965) because of asynapsis and anomalous multivalent formation. On the other hand, hybrids between *Trimerotropis maritima* and *T. citrina* are fertile (Carothers, 1941). Crosses have also been made between chromosomal races of the stick insect, *Didymuria violescens* (Craddock, 1971). The hybridization studies of White, Mrongovius and Craddock involve chromosomally differentiated and parapatrically distributed taxa and hence are particularly relevant to the study reported here. Studies of this sort are necessary for assessing the role of the chromosomal rearrangements in maintaining the integrity of the taxa.

Experimental hybridization of the "Moreton" and "Torresian" races of *Caledia* was performed for the following reasons:-

1) In order to test the introgression hypothesis derived from field data (see Chapter II), it was necessary to verify first that inter-racial hybridization could occur, and secondly that the hybrids were at least partially fertile. Additionally, the results of the hybridization could provide an explanation for the observed differences in the frequencies of presumed introgressed chromosomes both within "Moreton" populations and between "Moreton" populations fixed for either the acrocentric or metacentric X chromosomes (Chapter III).

2) The experiment could give some indication of the existence and nature of both pre-mating and post-mating isolating barriers between the two chromosomal races.

3) Meiosis could be examined in any hybrid males which were produced and an assessment made of chromosomally derived infertility (reported in Chapter V).

The design of the hybridization experiment was influenced by several factors including aspects of the breeding behaviour and biology of grasshoppers in general as well as limitations of time and facilities. Thus although single pair matings would provide the most useful information for statistical comparison between experimental crosses and controls, they are generally found to be inadequate in practice in Acridid grasshoppers because they have a lower level of productivity than bulk matings (Shaw, pers. comm.). Further, because of the labour intensive nature of grasshopper rearing, the time required for maintaining single pair matings for a comprehensive series of crosses would be prohibitive. The experiment was therefore carried out as a series of mass crosses using approximately 20 males and 20 females in each cross. In any case, the experimental design precluded a statistical analysis of the egg pod and progeny production because standard errors could not be calculated for any of the comparative parameters. Nevertheless useful inferences can be drawn from the data, where there are large and consistent differences, because the crosses were set up under similar conditions.

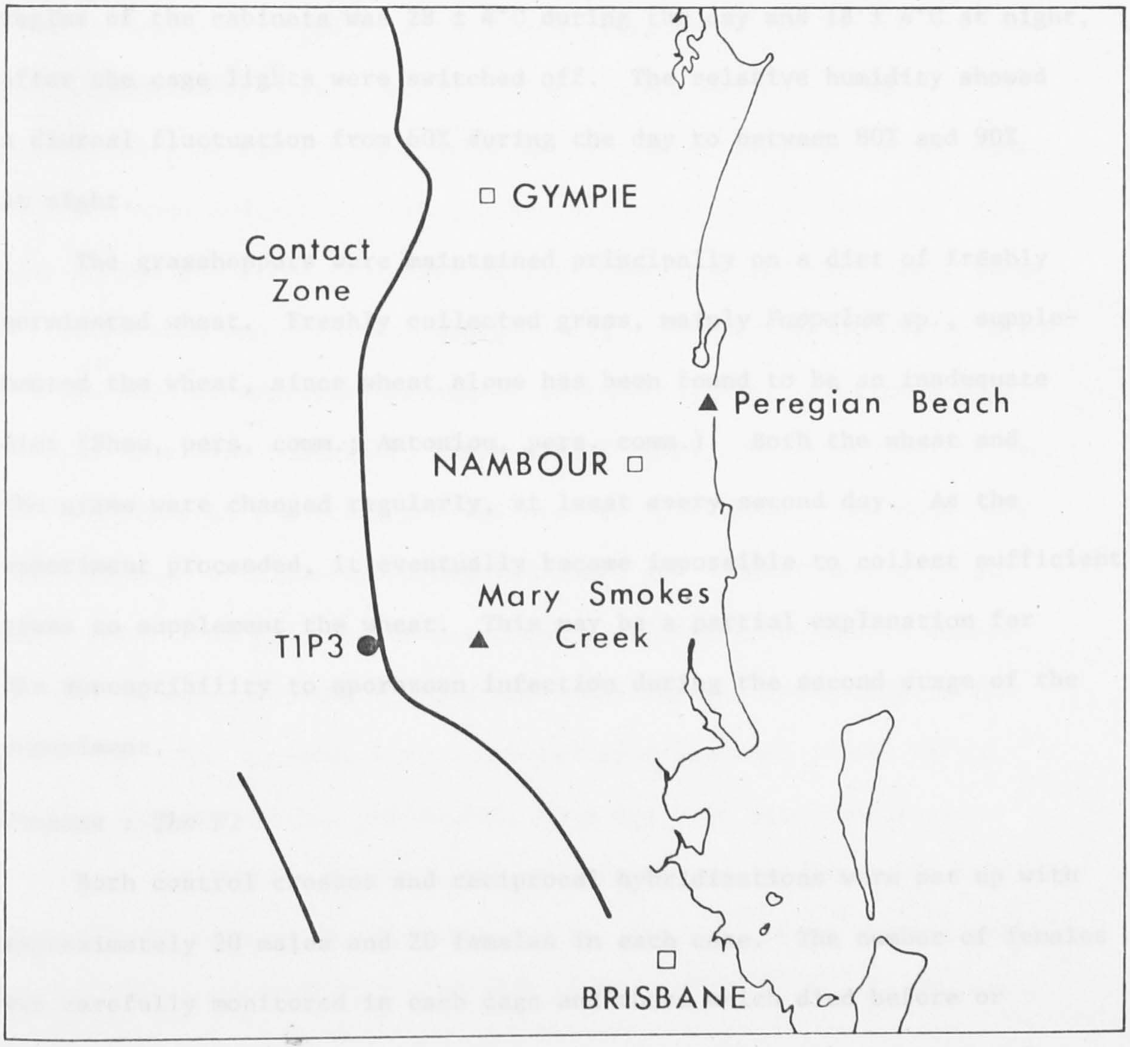
The "Moreton" populations which provided the parents for the crosses were chosen because they were known to be fixed for the two alternative X chromosomes. They were also chosen for their lack of autosomal polymorphism in order to simulate the expected conditions of hybridization when the races initially established parapatry, before any introgression had taken place. However, no precautions could be made to control the structural variation for chromosomes 7, 8 and 11 because of the presumed indigenous origin of these polymorphisms (see Chapter II).

MATERIALS AND METHODS

In January 1977, specimens of both races were collected in the field for use in laboratory crosses. At each of the three collection sites (see Figure 4.1), at least 60 males and 60 females were taken. Of the females, 40 nymphs were captured in each area to provide the virgin females necessary for the controlled hybridization. The virgin females were maintained in separate cages until they were used in the inter- and intra-racial crosses. Because of the difficulties encountered in collecting a sufficient number of female nymphs at any one collecting site, it was necessary to use adult females in the control crosses. Both nymphal and adult males were collected at the same sites.

The crosses were set up in grasshopper cages with internal dimensions of 35 cm x 37 cm x 48 cm. The sides and top of the cages consisted of fine wire mesh supported on a wooden frame. Access to the cage was via

Figure 4.1 Locations of the three populations used in the hybridization experiment.


□ GYMPIE
Contact Zone
▲ Peregian Beach
NAMBOUR □
Mary Smokes Creek ▲
T1P3 ●
□ BRISBANE

a cloth sleeve on the door of the cage. The sleeved allowed easy entry to the cage and prevented grasshoppers from escaping. Pots filled with damp sterile sand were placed in holes in the floor of the cage to provide egg laying sites. A 75W photoflood bulb was fixed to the top of each cage and a 14/10 light-dark regime was provided by automatic time switch.

Because of the danger of circulating allergenic dust from the grasshoppers, the cages were isolated in air conditioned cabinets. Masks were worn when handling the grasshoppers and cages. The temperature regime of the cabinets was 28 ± 4°C during the day and 18 ± 4°C at night, after the cage lights were switched off. The relative humidity showed a diurnal fluctuation from 60% during the day to between 80% and 90% at night.

The grasshoppers were maintained principally on a diet of freshly germinated wheat. Freshly collected grass, mainly *Paspalum* sp., supplemented the wheat, since wheat alone has been found to be an inadequate diet (Shaw, pers. comm.; Antoniou, pers. comm.). Both the wheat and the grass were changed regularly, at least every second day. As the experiment proceeded, it eventually became impossible to collect sufficient grass to supplement the wheat. This may be a partial explanation for the susceptibility to sporozoan infection during the second stage of the experiment.

Crosses : The F1

Both control crosses and reciprocal hybridizations were set up with approximately 20 males and 20 females in each case. The number of females was carefully monitored in each cage and those which died before or during the ultimate moult were subtracted from the total when egg pod and progeny production per female was calculated. Survival in general was high. The crosses were terminated when only five living females remained, since egg pod production was by then almost completed.

The F2

These crosses were set up under the same conditions as previously, with approximately 20 males and 20 females per cage. No backcrosses were attempted because of the insufficient number of grasshoppers and the lack of spare cages. Mortality was very high both in the control and hybrid individuals. A large number of grasshoppers died before the ultimate moult, due to the effects of sporozoan infection and egg pod production in the survivors was reduced. Reduced egg pod production is a recognized symptom of high levels of infection of females by the sporozoan *Malamoeba* sp. However, these infections do not depress the hatchability of the egg pods which are produced (Antoniou, pers. comm.).

The sand containers were checked daily for the presence of egg pods during the first two weeks of the experiment and then every second day in alternation with the feeding cycle. Care was taken not to break the egg pods or disturb them unduly during checking. When pods were present in a sand container, the number of pods, the cross and the date were recorded on the container and also on file cards. The pods were then dampened with sterile distilled water and covered with a glass petri dish. The glass cover prevented dessication, helped to reduce fungal contamination and trapped the newly emerged hatchlings in the sand pots. The egg pods were incubated at a constant 30°C. At the time of maximum egg pod production, some egg pods were temporarily stored in the rearing cabinets because of the lack of available incubator space. This delayed the development, but did not affect the hatchability, of the pods. A daily check was made on the pods and they were watered when necessary.

Approximately 28 days after laying (for pods maintained at 30°C), the first progeny hatched from the pods. The number of emerging hoppers was recorded on the appropriate file cards. The sand pots were returned to the incubator to ensure complete hatching of the pod. The hatchlings were initially raised in small cages and subsequently transferred to larger cages to minimize overcrowding. Mortality was high in the first and second instar but approximately the same in both control and hybrid progeny.

TABLE 4.1

Site Name	Race	Sex Chromosome	Autosomes
T1P3	"Torresian"	Acrocentric	Very low frequency of introgressed autosomes (Chapter III)
Peregian Beach	"Moreton"	Acrocentric	Very little polymorphism - population 3 in Table 3, Chapter II.
Mary Smokes Creek	"Moreton"	Metacentric	Some polymorphism - estimate from populations 19,20,21 and 25 in Table 3, Chapter II

TABLE 4.2

X Males Females	"Torresian"	"Moreton" acrocentric X	"Moreton" metacentric X
Torresian	Control	Inter-racial F1	Inter-racial F1
Moreton acrocentric X	Inter-racial F1	Control	Intra-racial F1
Moreton metacentric X	Inter-racial F1	Intra-racial F1	Control

Pattern of experimental hybridization to produce both inter- and intra-racial F1 hybrids. The F2 was produced by crossing male and female products from each of the cells of this matrix.

RESULTS

The F1. A total of 527 control and hybrid egg pods (see Table 4.3) were produced in the first stage of the experiment, in the period from 18/1/1977 to 22/3/1977. These pods produced their first hatchlings in the period from 16/2/1977 to 27/4/1977, although hatching was not completed in some pods up to two months later. The total number of hatchlings in the first laboratory generation was 6,015. Hatchability was generally good, although in both controls and inter- and intra-racial crosses, some pods gave very few or no progeny. These pods were generally found to be infected by a fungus, although it was not clear whether the fungus was pathogenic or was merely a saprophyte growing on eggs which died from other causes. There are no clear and consistent differences between the inter-racial crosses and parental controls, particularly in terms of the average number of progeny per pod. Here the largest differences occur between the reciprocal inter-racial crosses.

TABLE 4.3

"Moreton" meta X Control

No. of egg pods = 79 Av. no. progeny/pod = 13.58
No. of progeny = 1,073 Av. no. pods/female = 3.59
No. of females = 22 Av. no progeny/female = 48.73

"Moreton" acro X Control

No. of egg pods = 86 Av. no. progeny/pod = 12.66
No. of progeny = 1,089 Av. no. pods/female = 4.53
No. of females = 19 Av. no. progeny/female =57.32

"Torresian" Control

No. of egg pods = 70 Av. no. progeny/pod = 12.75
No. of progeny = 893 Av. no. pods/female = 2.92
No. of females = 24 Av. no. progeny/female =37.21

"Torresian" Females x "Moreton" acro X Males

No. of egg pods = 22 Av. no. progeny/pod = 14.00
No. of progeny = 308 Av. no. pods/female = 1.57
No. of females = 14 Av. no. progeny/female =22.00

"Moreton" acro X Females x "Torresian" Males

No. of egg pods = 22 Av. no. progeny/pod = 6.27
No. of progeny = 138 Av. no. pods/female = 1.47
No. of females = 15 Av. no. progeny/female = 9.20

"Torresian" Females x "Moreton" meta X Males

No. of egg pods = 51 Av. no. progeny/pod = 14.00
No. of progeny = 714 Av. no. pods/female = 2.55
No. of females = 20 Av. no. progeny/female =35.70

"Moreton" X Females x "Torresian" Males

No. of egg pods = 79 Av. no. progeny/pod = 8.15
No. of progeny = 644 Av. no. pods/female = 3.95
No. of females = 20 Av. no. progeny/female =32.20

"Moreton" meta X Females x "Moreton" acro X Males

No. of egg pods = 32 Av. no. progeny/pod = 9.53
No. of progeny = 305 Av. no. pods/female = 1.68
No. of females = 19 Av. no. progeny/female =16.05

"Moreton" acro X Females x "Moreton" meta X Males

No. of egg pods = 86 Av. no. progeny/pod = 9.90
No. of progeny = 851 Av. no. pods/female = 4.53
No. of females = 19 Av. no. progeny/female =44.79

F1 data. The only figures which can be compared between crosses are those in the right hand column.

The F2. On the 9/5/1977, the control and F1 hybrid crosses in the second stage of the experiment were commenced and continued until the 21/7/1977. In this period, 92 egg pods were produced. This is almost a six-fold reduction in egg pod production compared with the previous stage of the experiment. In general, the control crosses showed a greater depression of egg pod production than the hybrid crosses (Table 4.4), principally because of disease. The "Moreton" metacentric X control failed to produce any normal pods.

The F2 and L2 pods hatched in the period from 9/6/1977 to 26/7/1977. There was a high rate of failure of pods to produce any hatchlings. Both types of reciprocal F1 hybrids derived from crosses between "Torresian" and "Moreton" metacentric X individuals failed to produce any F2 progeny from 12 pods. For the "Torresian" and "Moreton" acrocentric X reciprocal F1 hybrid crosses, progeny were obtained from only 13 of the 35 egg pods and even then the number was severely depressed.

The control and intra-racial crosses produced F2 pods with considerably better hatchability but even so, 5 pods from a total of 45 failed to give any progeny at all.

Overall a total of only 506 progeny were obtained from the second stage of the experiment, which is approximately a 12-fold reduction in progeny production compared with the original crosses. This can be attributed to disease which affected egg pod production in both hybrids and parental controls and more importantly to the failure of the inter-racial F2 hybrid pods to produce a similar number of progeny to the F1 hybrid pods. The depression in the average number of progeny produced per F2 inter-racial hybrid pod is very severe (Table 4.4), particularly when one considers that the pods were normal in shape and size and appeared to contain a normal number of eggs.

There was a high rate of mortality as usual in the first and second instar nymphs in both F2 hybrids and controls. None of the F2 inter-racial hybrid progeny survived to adulthood, so chromosomal segregation and male meiosis has not been examined in them. However, the data are sufficient to allow some conclusions to be drawn from them concerning isolating mechanisms between the races and the pattern of polymorphism in the "Moreton" race.

TABLE 4.4.

"Moreton" meta X Control

No. of pods = 1 *Protein tube only
No. of progeny = 0

"Moreton" acro X Control

No. of pods = 4 Av. no. progeny/pod = 9.25
No. of progeny = 37

"Torresian" Control

No. of pods = 10 Av. no. progeny/pod = 15.40
No. of progeny = 154

("Torresian" Females x "Moreton" acro X Males) F1 X F1

No. of pods = 25 Av. no. progeny/od = 1.92
No. of progeny = 48

("Moreton" acro X Females x "Torresian" Males) F1 X F1

No. of pods = 10 Av. no. progeny/pod = 1.40
No. of progeny = 14

("Torresian" Females x "Moreton" meta X Males) F1 X F1

No. of pods = 1 Av. no. progeny/pod = 0
No. of progeny = 0

("Moreton" meta X Females x "Torresian" Males) F1 X F1

No. of pods = 11 Av. no. progeny/pod = 0
No. of progeny = 0

("Moreton" meta X Females x "Moreton" acro X Males) F1 X F1

No. of pods = 18 Av. no. progeny/pod = 8.17
No. of progeny = 147

("Moreton" acro X Females x "Moreton" X Males) F1 X F1

No. of pods = 12 Av. no. progeny/pod = 8.83
No. of progeny = 106

F2 data. Because of the reduced production of egg pods, only the average number of progeny per pod can be derived.

*A protein tube is normally laid down around an egg mass to form an egg pod. In this case, the protein tube contained no eggs.

DISCUSSION

The crossing experiments have clearly demonstrated that hybridization is possible between the "Moreton" and "Torresian" races of *Caledia captiva* and that viable F1 progeny are produced. Although precise statistical comparisons cannot be made, it is clear that the productivity of both of the reciprocal crosses between "Torresian" and "Moreton" metacentric X individuals was as high as the control levels for most of the measured parameters (Table 4.3). Moreover, it was considerably better than that found in the intra-racial cross between "Moreton" metacentric X females and "Moreton" acrocentric X males. These data provide convincing support for the interpretation of the data from Transect 1 (Chapter III), where it was concluded, on the basis of the frequencies of the parental chromosomal types and F1 hybrids, that random mating was taking place in the contact zone with normal survival of any hybrids. On the other hand, both egg pod production per female and progeny production per female were considerably depressed below control levels in both of the reciprocal crosses between "Torresian" and "Moreton" acrocentric X individuals. Even the average number of progeny per pod of 6.27 for the cross between "Moreton" acrocentric X females and "Torresian" males is only half the value for the "Moreton" acrocentric X control, although the average number of progeny per pod from the reciprocal cross of 14.00 exceeds the "Torresian" control level. Hatchability of the egg pods may also have been affected in the former case. However a similar pattern of reciprocal difference was observed in the crosses between "Moreton" metacentric X and "Torresian" individuals (Table 4.5). The differences between the crosses may be an expression of maternal effect, since "Torresian" egg pods have previously been found to produce a higher average number of progeny than "Moreton" egg pods (Shaw, pers. comm.), although this difference was not observed in this experiment.

The results of the intra-racial crosses are anomalous. The cross between "Moreton" acrocentric X females and "Moreton" metacentric X males has performed as well as both the "Moreton" controls in terms of egg pod and progeny production per female. Both intra-racial crosses, however, have a lower average number of progeny per pod with 3 to 4 hatchlings on average less than the controls. In this respect, they resemble the two crosses between "Moreton" females and "Torresian" males. The cross between "Moreton" metacentric X females and "Moreton" acrocentric X males also produced considerably fewer egg pods and progeny per female than either of the "Moreton" controls. A possible explanation for these differences between the reciprocal intra-racial crosses can be found in the larger size of the "Moreton" acrocentric X grasshoppers, particularly the females (see Chapter VI), which accentuates the normal sexual dimorphism in crosses where they are used as the female parent and diminishes the dimorphism when they are used as the male parent. This may modify the normal mating behaviour.

The number of progeny per pod is the most reliable of the three derived parameters in Table 4.3. Many unknown factors can influence the production of egg pods and hence the number of progeny produced by each female. In particular, because of the experimental design, it is not known whether all of the adult females are contributing equally to egg pod production in each of the experimental cages. The actual number of egg pod producers cannot be deduced in these experiments and the potential number must be used in all calculations of female productivity.

The F1 data do not provide an explanation for the higher rate of introgression of specific "Torresian" chromosomes into "Moreton" acrocentric X populations than into "Moreton" metacentric X populations (see also Chapter III). If anything, the results are the reverse of what one might expect. There is in general a lower productivity in hybridization between "Torresian" and "Moreton" acrocentric X individuals despite the higher level of introgression of some "Torresian" chromosomes into the "Moreton" acrocentric X populations. However, given the chromosomally specific nature of the introgression, the F1 data could only provide a partial explanation for these results, since differential survival of individual chromosomes must take place in later generations derived from the F1.

The F2 data indicate severe hybrid breakdown in all of the inter-racial hybrids (Table 4.4), even though the "Moreton" metacentric X control failed to produce any normal egg pods. In this control cross, the single egg pod consisted of a protein tube without any eggs. However the failure of egg pod production in this case can be attributed to the effects of disease. The sporozoan, *Malamaeba*, affected the L1 generation of this control so severely that very few individuals survived to adulthood, and the few which did, failed to survive long enough to produce egg pods. A clear distinction must be made between the failure of progeny production in this case and the failure of the inter-racial F2 generation. The performance of the "Torresian" and "Moreton" acrocentric X controls, as well as that of the "Moreton" intra-racial hybrids provides a good standard to allow comparison with the performance of the inter-racial hybrids.

The cross between hybrids derived from the mating of "Torresian" females and "Moreton" metacentric X males produced only one F2 pod. The other three categories of inter-racial F1 hybrids, on the other hand, produced more egg pods than the controls and were about as productive as the intra-racial F1 hybrids in this regard (Table 4.4). The hatchability data are therefore more reliable for the inter-racial hybrids than for the controls. The breakdown appears to be more severe in the F2 derived from "Moreton" metacentric X-"Torresian" F1 hybrids, where no F2 progeny were obtained from 12 egg pods, than in the F2 derived from "Moreton" acrocentric X-"Torresian" F1 hybrids, although it is difficult to estimate the exact magnitude of this difference. In any case, the less severe effect of hybrid breakdown in the F2 derived from "Moreton" acrocentricX-"Torresian" hybrids is concordant with the higher frequency of introgression of "Torresian" autosomes into "Moreton" populations fixed on the acrocentric form of the X chromosome (Chapters II and III). Assuming that adverse epistatic interactions are important in preventing the introgression of certain "Torresian" autosomes, these F2 data support the hypothesis that there are fewer possible unfavourable interactions in "Moreton" acrocentric X populations.

It should also be noted that the breakdown in the production of inter-racial F2 hybrid progeny is too severe to be explained by the meiotic anomalies observed in the F1 hybrids (see Chapter V). At worst, the observed meiotic anomalies would cause a reduction of 50-60% in progeny production in the F2. However this assumes that all aneuploid gametes would give rise to zygotes which were inviable at an early stage of development and that there was no elimination of unbalanced meiotic cells in polyploid spermatids in males. In this experiment there is a 100% reduction in the production of the "Moreton" metacentric X-"Torresian" F2 progeny and an estimated reduction of 75% to 85% in the production of the "Moreton" acrocentric X-"Torresian" F2. Not only are these values too high for the predictions based on the frequency of meiotic anomalies in the F1, but they are also in the wrong order. The "Moreton" metacentric X-"Torresian" F1 hybrids had a lower level of meiotic anomalies than the "Moreton" acrocentric X-"Torresian" F1 hybrids, but had a larger reduction in the production of F2 progeny.

The isolation estimate parameter of Merrel (1950) provides a concise means of summarizing the control and hybrid data (Table 4.5). In this case, the production data are summarized to allow a simple comparison between the intra-racial and inter-racial crosses and also between the F1 and F2 progeny per pod data. The F1 isolation estimates indicate that at least in terms of progeny production per pod, which is the most reliable parameter, the inter-racial crosses have performed as well as, if not better than, the intra-racial crosses. Indeed, all three "Moreton" metacentric X and "Torresian" F1 isolation values are larger than the corresponding intra-racial values. The "i" estimate of 0.998 for the number of pods per female is close to the expected value of 1, when the hybrid crosses perform as well as the controls.

TABLE 4.5

	Comparison		
"i" estimate based on:	"Moreton" metacentric X, "Torresian"	"Moreton" acrocentric X, "Torresian"	"Moreton" metacentric X, "Moreton" acrocentric X
F1 Pods/female	0.998	0.408	0.765
F1 Progeny/female	0.790	0.330	0.574
F1 Progeny/pod	0.841	0.798	0.740
F2 Progeny/pod	0	0.135	0.745

Isolation estimates derived from both F1 and F1 data.

The "i" parameter $\left(i = \frac{(A \times B) + (B \times A)}{(A \times A) + (B \times B)} \right)$ was originally devised by Merrel (1950) as an estimate of behavioural isolation but applies to breeding data in this case. A value of 1 indicates no isolation and a value of 0 indicates complete isolation.

The F2 isolation estimates demonstrate the extent of the breakdown in the inter-racial F2 hybrids. In the calculation of these values, it was necessary to use the F1 progeny per pod figure for the "Moreton" metacentric X control to avoid grossly inflating the intra-racial "i" value. Nevertheless there is still a very large difference between the intra-racial and inter-racial isolation values. The intra-racial values are consistent for both the F1 and F2 data, but there is a very large decrease for the inter-racial comparisons between the F1 and F2. In other words, the postmating isolating mechanism between the "Torresian" and "Moreton" races does not affect the F1 hybrids but acts on subsequent generations.

CHAPTER V

Cytological Analysis of Hybrids

INTRODUCTION

The cytological analysis of hybrids between karyotypically differentiated taxa is important in two respects. First, even the examination of mitosis in hybrid cells with a common cytoplasmic background extends the detailed comparisons of chromosome morphology, which may only be inferred from studies of the chromosomes of the parental types. In particular, an accurate comparison can be made of the size of presumptive homologous chromosomes, which is not impeded by the difficulty of obtaining "pure" cells with exactly the same state of chromosome contraction. Similarly, the banding pattern of each haploid genome can be compared within the same cell. The power of these comparative mitotic chromosome assays is, of course, greatly enhanced by including an examination of meiosis in the hybrids, where the pairing relationship of the chromosomes or chromosome segments allows confirmation or rejection of the homology suggested solely on the basis of the mitotic analyses.

Secondly, cytological examination of meiosis in hybrids permits an assessment of the fertility of these hybrids after a detailed analysis of chromosome pairing and segregation patterns. The importance of such factors as asynapsis, desynapsis, crossing over in inverted segments and malorientation of multivalents can be quantified, thus allowing an estimation to be made of their effective fertility. If a sufficiently detailed comparison is made, it may be feasible to attribute the anomalies either to the structural differences in the chromosomes or to genic incompatibilities between the taxa. This particular distinction is obviously important when attempts are made to determine the mode of speciation of the taxa involved.

Cytological examination of hybrids between closely related taxa from several distinct groups in the animal kingdom has been frequently reported in the literature. In some cases, the taxa concerned were karyotypically distinct, although often morphologically similar, while in other cases, the karyotypes have been similar and the taxa have been distinguished on external morphology. Thus, in the newts, male meiosis has been examined in hybrids between *Triturus cristatus* and *T. marmoratus* (White, 1946) and in hybrids between geographical races of *Triturus cristatus* (Callan and Spurway, 1951). None of the taxa concerned can be distinguished mitotically. Asynapsis and the occasional formation of multiples, which was originally interpreted as evidence for interchanges, were the only class of anomalies detected, but the spermatids were found to degenerate, leading to sterility of the males. Similar studies on the karyotypically distinct taxa of *Mus musculus*, where both male and female meiosis have been examined, demonstrated that the hybrid sterility was due to malorientation of the very large chain and ring multivalents in the hybrids (Capanna *et al.*, 1976). Asynapsis and malorientation of multivalents have also been demonstrated in hybrids between taxa of the *Didymuria violescens* complex (Craddock, 1971).

Grasshopper hybrids have been particularly well studied in this regard because of their relatively large chromosomes and the ease with which the meiosis of the males can be examined. Spermatogenesis has been analyzed in F1 hybrids of *Chorthippus bicolor* and *Ch. biguttulus* (Klingstedt, 1939) and revealed normal pairing, but a breakdown of meiosis at anaphase 1. The hybrids of *Circotettix verruculatus* and *Trimerotropis suffusa* (Helwig, 1955) show a highly variable level of asynapsis. Univalent formation, malorientation of multivalents and the occurrence of anomalous multiples were detected in hybrids of the 15 and 17 races of *Keyacris (Moraba) scurra* (White, 1957). Hybrids of *Eyprepocnemis plorans plorans* and *E. p. ornatipes* (John and Lewis, 1965) have been examined and revealed a high frequency of anomalies, including asynapsis and multiple formation. Three races in Viatica group of Morabine grasshoppers have been hybridized. Although male hybrids of

P(24 XY) and *viatica* 19 appear to be inviable, the two other classes of male hybrids are viable and meiosis has been examined. For *viatica* 17-*viatica* 19 hybrids, it was estimated that 20-25% of the gametes were aneuploid as a result of meiotic anomalies. On the other hand, the level of asynapsis in P(24 XY)-*viatica* 17 hybrids was only slightly higher than in the parental controls (Mrongovius, 1975). In *Caledia captiva*, the "Daintree" race has been crossed with the remaining three races and hybrids between the "Torresian" and "South east Australian" races have been produced (Shaw, pers. comm.). The level of anomalies ranges from a low level of asynapsis in the "Moreton" - "South east Australian" F1 and F2 hybrids to complete inhibition of meiosis in hybrids between the "Daintree" and "Torresian" races.

Field collected hybrids between closely related taxa have also been examined. They are of particular relevance to this study when the hybrids are collected in the contact zone between parapatric taxa. For example, hybrids collected in the contact zones of the Viatica group of Morabine grasshoppers (White *et al.*, 1969; Mrongovius, 1975) and hybrids from some of the contact zones between the chromosomal races of *Didymuria violescens* (Craddock, 1971) have been examined cytologically and compared with the laboratory hybrids. Surprisingly, the field hybrids have generally been found to have a lower level of meiotic anomalies than the laboratory produced hybrids. A field collected hybrid between the sympatric species, *Trimerotropis thalassica* and *T. occidentalis*, has also been examined by John and Weissman (1977), who found it had meiotic anomalies attributed to the tandem translocation difference between the species.

In this study, both field collected (Chapter III) and laboratory produced hybrids (Chapter IV) of the "Moreton" and "Torresian" races have been analyzed. Conventional mitotic preparations from mid gut caeca and C-banded preparations of embryonic material from laboratory reared hybrids and controls were also examined. Furthermore, a quantitative analysis of the level of meiotic anomalies at metaphase 1 has been performed on the hybrids to assess the role of hybrid infertility in maintaining the integrity of the taxa. Pre-mating behavioural isolating mechanisms and two postmating isolating mechanism, namely sperm reactions and hybrid inviability, have already been eliminated as important contributors to the inter-racial isolation (see Chapters III and IV). F1 infertility and hybrid breakdown remain as two possible mechanisms which may maintain the distinctness of the taxa and the narrowness of the hybrid zone. Meiotic analysis provides an excellent means of distinguishing between the two categories.

MATERIALS AND METHODS

Hybrid grasshoppers were collected in the field during the detailed sampling across the contact zone between the two chromosomal races (Chapter III) and meiosis was examined in the males. F1 hybrids together with parental controls were also reared in the laboratory (Chapter IV). These were examined using three methods:-

(1) C banded embryonic mitosis
(2) Orcein stain C-mitosis
(3) Meiotic analysis

1) C banding

Eight-day-old embryos were carefully dissected out and placed in 0.05% colchicine in insect saline for 2 hours. They were then fixed in 3:1 methanol:acetic acid for 1 hour. Air dried preparations were made on slides (precleaned in chromic acid) by macerating the embryos in a drop of 60% acetic acid. The drop was moved around to distribute the cells and the slide was allowed to dry. The slides were stored overnight on a warming plate at about 50°C. They were then treated with saturated $Ba(OH)_2$ for 7 minutes, rinsed in distilled water and incubated in 2x SSC at 65°C for 90 minutes. After rinsing in distilled water, the slides were stained with 10% Giemsa (Gurr's Improved R66) for 30 minutes and allowed to dry. After a preliminary check to determine the success of the staining procedure, coverslips were mounted using XAM neutral mountant or the staining procedure was repeated.

2) *Orcein stained mitosis*

Orcein stained preparations of mid gut ceaecal material were made according to the techniques reported in Chapter II.

3) *Meiotic analysis*

Testes were dissected from males and fixed in 3:1 ethanol:acetic acid. Squash preparations were stained with lactopropionic orcein. A quantitative analysis of anomalies at metaphase 1 was made. Anomalous cells at other stages of meiosis were noted but not scored. A representative sample of anomalies at all stages of meiosis was photographed.

RESULTS

1) *Field hybrids from Transect 1*

Ten F1 hybrid males were collected in the contact zone between the "Moreton" and "Torresian" races and all were examined in detail to determine the level of meiotic anomalies. Two categories of male hybrids must be recognized on the basis of X chromosome morphology. These are:-

(1) Individuals carrying a "Moreton" metacentric X chromosome, which are derived from the cross between "Moreton" females and "Torresian" males.

(2) Individuals with a "Torresian" acrocentric X chromosome. These result from the reciprocal cross involving "Torresian" females and "Moreton" males.

The main category of anomalies found in the hybrids was the presence of univalents at metaphase 1. Two other types of anomalies were also observed (see Table 5.1). These were tetraploid metaphase 1 cells found only in the "Moreton" control and multivalents restricted to the hybrids.

TABLE 5.1

		Anomalies						
Categories	No. Indiv.	0	2	4	6	IV	Tetra	No. Cells
"Moreton" meta X	6	534	13	0	0	0	4	551
Control		96.9	2.4				0.7	%
"Torresian"	4	336	3	0	0	0	0	339
Control		99.1	0.9					%
F1 hybrid	10	750	81	8	1	5	0	845
combined		88.8	9.6	0.9	0.1	0.6		%
F1 hybrid	3	208	14	3	0	0	0	225
meta X		92.5	6.2	1.3				%
F1 hybrid	7	542	67	5	1	5	0	620
acro X		87.4	10.8	0.8	0.2	0.8		%
F1 acro X	4	291	10	0	0	1	0	302
-low asynapsis		96.4	3.3			0.3		%
F1 acro X	3	251	57	5	1	4	0	318
- high asynapsis		78.9	17.9	1.6	0.3	1.3		%

Frequency of meiotic anomalies in field collected hybrid and controls

If we ignore these two minor classes of anomaly, the remaining asynapsis data can be tested for heterogeneity. The "Torresian" control data appear to be heterogeneous, since all three univalent containing cells were observed in the one individual, but it is not possible to apply a statistical test in this case. The data for the "Moreton" metacentric X control individuals are also highly heterogeneous and again the heterogeneity can be attributed to a single individual, which contained 9 univalent bearing cells out of a total of 111. The pooled F1 hybrid data are extremely heterogeneous (Table 5.2). Even when the metacentric X and acrocentric X hybrids are analyzed separately, both groups are still significantly heterogeneous, although in the former case it is only at the 5% level. The acrocentric X hybrid data, on the other hand, are highly significantly heterogeneous. These data can be divided into a homogeneous group of 4 low asynaptic individuals, in which a mean of 3.3% of the cells contain univalents and a heterogeneous group of 3 highly asynaptic individuals, in which a mean of 19.8% of the cells contained univalents.

TABLE 5.2

	Mean Asynapsis	Range	Heterogeneity
"Moreton" meta X Control	2.38	0-8.82	22.95 (5) ***
"Torresian" Control	0.88	0-4.61	-
F1 hybrid combined	11.99	1.27-36.36	65.28 (10) ***
F1 hybrid meta X	8.89	2.25-17.10	8.89 (3) *
F1 hybrid acro X	13.13	1.27-36.36	49.94 (6) ***
F1 acro X - low asynapsis	3.32	1.27-5.26	1.95 (3) N.S.
F1 acro X - high asynapsis	22.47	14.48-36.36	11.26 (4) *

Mean and range of frequency of asynapsis in field collected hybrids and controls * $P < 0.05$ *** $P < .005$

In the "Torresian" and "Moreton" controls, the frequency of asynaptic cells was 0.9% and 2.4% respectively. On the other hand, the frequency of asynaptic cells in the F1 hybrids ranges from 3.3% in the low asynaptic acrocentric X hybrids up to 22.3% for the highly asynaptic hybrids (Table 5.1). The highest frequency of asynapsis per individual was 34.9% and was found in an acrocentric X hybrid. The overall mean frequency of asynaptic cells for the pooled hybrids was 11.9%. However in spite of the difference in the average value between hybrids and controls, half of the field collected hybrids fall within the range of anomalies in the controls. In other words, fertility depression in the hybrids is unlikely to play a major role in keeping these taxa separate.

2) A control study of the level of meiotic anomalies in structurally heterozygous individuals from the "Moreton" race

Meiosis was examined in previously mitotically characterized males from the Spring Valley Creek population (Chapter II) and from T1PB (Chapter III). The males were chosen because they were known to be structurally heterozygous for one, or at most two, identified chromosome pairs. The aim of the analysis was to determine whether the structural heterozygosity caused a higher level of meiotic anomalies, particularly the occurrence of univalency at metaphase 1, in these particular pairs.

Four hundred and twenty nine (429) cells were scored from 11 individuals from these two populations which are fixed for the acrocentric or metacentric forms of the X chromosome. Univalents were found in only one cell in an individual from Spring Valley Creek. However the univalent chromosomes in this case were not members of a heteromorphic pair, but were the structurally homozygous acrocentric chromosome 7 pair. No asynapsis was observed in any of the structurally heterozygous pairs from either population (Table 5.3). The observed level of asynapsis in the heteromorphic pairs is lower than the level of asynapsis in the homomorphic pairs. Therefore it must be concluded that structural heterozygosity *per se* does not lead to an increase in the frequency of univalency or other meiotic anomalies.

TABLE 5.3

		Heteromorphic Pair					
Population		1	2	4	5	6	10
T1PB	No cells	54	54	52	35	35	53
meta X	No Univs	0	0	0	0	0	0
Spring Valley	No cells	171	129	54	-	171	116
acro X	No Univs	0	0	0	-	0	0

The frequency of asynapsis in individuals heteromorphic for specific, inidividual chromosome pairs.

3) C banded mitosis

The banding patterns of the "Moreton" and "Torresian" races have been shown to be strikingly different (Shaw, Webb and Wilkinson, 1976). It is therefore of interest to observe these differences in hybrid cells. A sample of parental embryos as well as both intra-racial and inter-racial hybrid embryos have been C banded, and the differences observed by the previous authors have been verified in reciprocal hybrids (Fig. 5.1, 5.2). The "Torresian" chromosomes show only a small centromeric C-banded region except for chromosomes 10, 11 and 12 which in addition have terminal bands. The terminal band on the "Torresian" chromosome 10 is smaller and not so darkly stained as the equivalent band on the "Moreton" chromosome 10 in hybrids.

The "Moreton" chromosomes differ strikingly in their pattern of banding from the "Torresian" chromosomes. In general, there are prominent

Figure 5.1 C-banded embryonic cells.

(a) "Moreton" metacentric X male.

(b) Intra-racial hybrid female from cross between "Moreton" acrocentric X females and "Moreton" metacentric males.

(c) Intra-racial hybrid female derived from the same cross as (b).

(d) Another cell from the same individual as (c).

(e) Male intra-racial hybrid derived from cross between "Moreton" acrocentric X females and "Moreton" metacentric X males.

(f) Male inter-racial Fl hybrid from cross between "Moreton" acrocentric X females and "Torresian" males.

(g) Male inter-racial Fl hybrid from cross between "Moreton" metacentric X females and "Torresian" males.

(h) Male inter-racial Fl hybrid from cross between "Torresian" females and "Moreton" metacentric X males.


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Figure 5.2 Karyograms of C-banded embryonic cells.

(Read in conjunction with Figure 5.1)

(a) Note metacentric X chromosome, structural heterozygosity for chromosome 1 and band heterozygosity for chromosome 9.

(b), (c), (d). Note structural heterozygosity for the X chromosome and the consistency of the banding patterns in these three cells.

(e) Intra-racial F1 hybrid male with an acrocentric X chromosome. Note the unbanded form of chromosome 9 in this "Moreton" individual, which closely resembles the "Torresian" 9.

(f) Note structural heterozygosity coupled with heterozygosity for presence/absence of C-bands in this inter-racial F1 hybrid. This individual also carries an unusual form of the "Moreton" chromosome 9, which has 4 C-bands, rather than 1.

(g) F1 hybrid male with metacentric X chromosome. This individual has a metacentric form of chromosome 5, which must have been derived from the "Torresian" parent. It also possesses two different acrocentric forms of chromosome 1.

(h) Inter-racial F1 hybrid male with "Torresian" X chromosome.


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interstitial and terminal C-bands on all chromosomes. However two "Moreton" individuals (Fig. 5.2a,e) carried forms of chromosome 9 which did not have the expected interstitial C-band, and thus resemble more closely the "Torresian" form of this chromosome. This has been interpreted as evidence for introgression of the "Torresian" chromosome 9 into the "Moreton" race. Because of the similarity of the unbanded forms of the "Torresian" and "Moreton" chromosome 9, introgression of this chromosome could not be reliably induced from the previous studies using conventional staining techniques (Chapter II).

A comparison of the parental complements in the F1 hybrids has shown that there are a minimum of 35 chromosomal differences between the "Moreton" and "Torresian" races when both C-banding and chromosome morphology are considered. This contrasts with the very low level of morphological divergence.

4) Conventional mitotic preparations of hybrids

Conventional mitotic preparations were made of all hybrid males on which meiotic analyses were performed. In addition mitosis was examined in a sample of hybrid females. Examples of all types of reciprocal hybrids have been photographed and are shown in Figures 5.3 and 5.4. These examples demonstrate the differences between the unbanded karyotypes of the "Moreton" and "Torresian" races within the cells of hybrids. In particular, chromosome 12 of the "Moreton" race usually has a visible short arm. Further it was noted that the short arm of the "Moreton" 12 could only be detected in very clear cytological preparations. Thus the admittedly imprecise estimates of the frequency of the acrocentric 12 in the "Moreton" race (Chapter II) probably severely underestimate the frequency of this morph. The slight difference in morphology between the telocentric "Torresian" chromosome 9 and the "Moreton" acrocentric form was also confirmed in the hybrids.

In hybrids between the "Moreton" metacentric X and "Torresian" individuals, a puzzling and as yet not fully resolved observation was made concerning chromosome 1. As expected, an acrocentric form of this chromosome, derived from a "Moreton" parent, was ocassionally found in

Figure 5.3. Conventional mitotic preparations of laboratory raised inter-racial hybrids.

(a) ("Moreton" metacentric X x "Torresian") Fl hybrid female

(b) ("Moreton" metacentric X x "Torresian") Fl hybrid female

(c) ("Moreton" metacentric X x "Torresian") Fl hybrid male

(d) ("Torresian" x "Moreton" metacentric X) Fl hybrid female

(e) ("Torresian" x "Moreton" metacentric X) Fl hybrid male

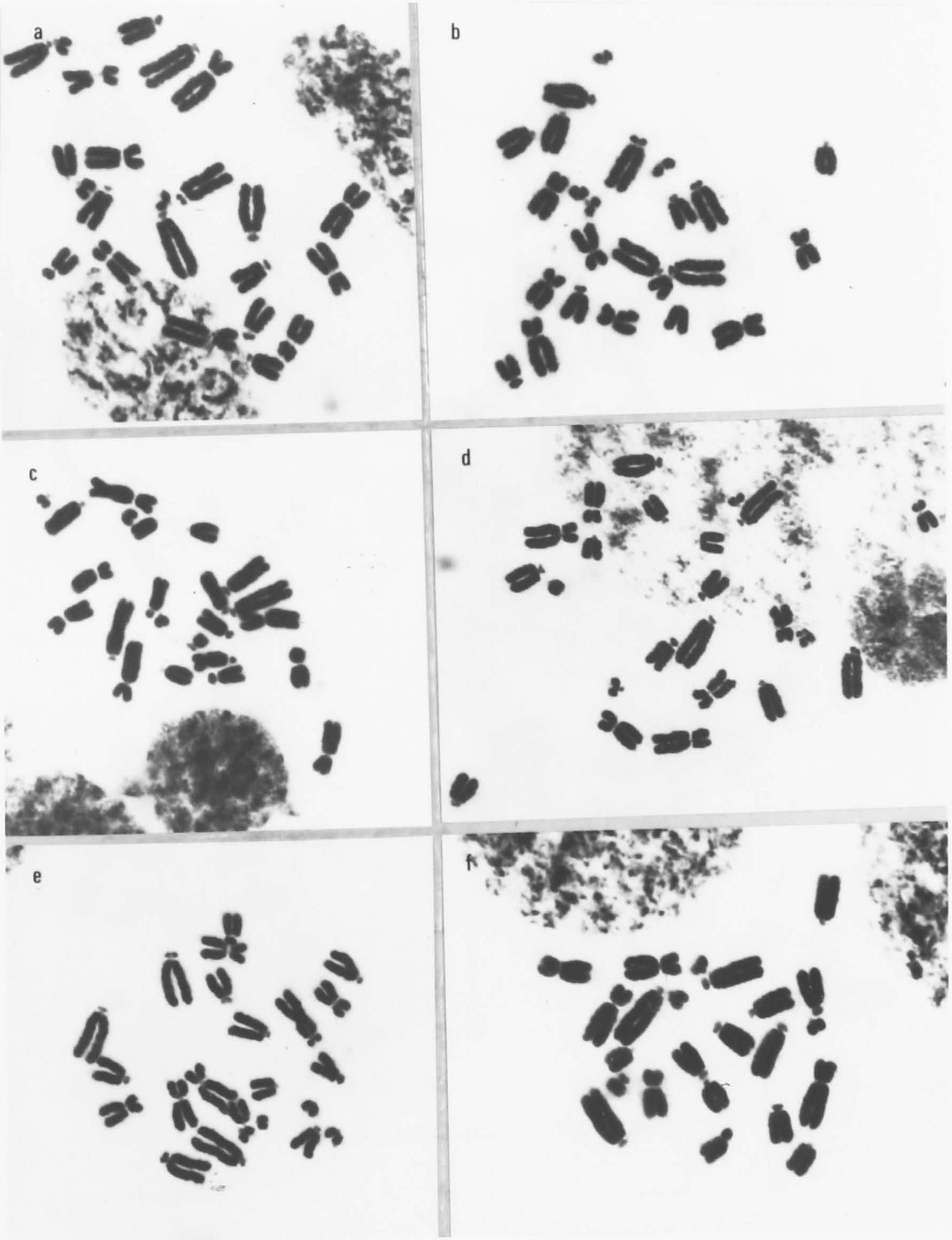
(f) ("Torresian" x "Moreton" metacentric X) Fl hybrid male

(g) ("Moreton" acrocentric X x "Torresian") Fl hybrid female

(h) ("Moreton" acrocentric X x "Torresian") Fl hybrid female

(i) ("Moreton" acrocentric X x "Torresian") Fl hybrid male

(j) ("Torresian" x "Moreton" acrocentric X) Fl hybrid male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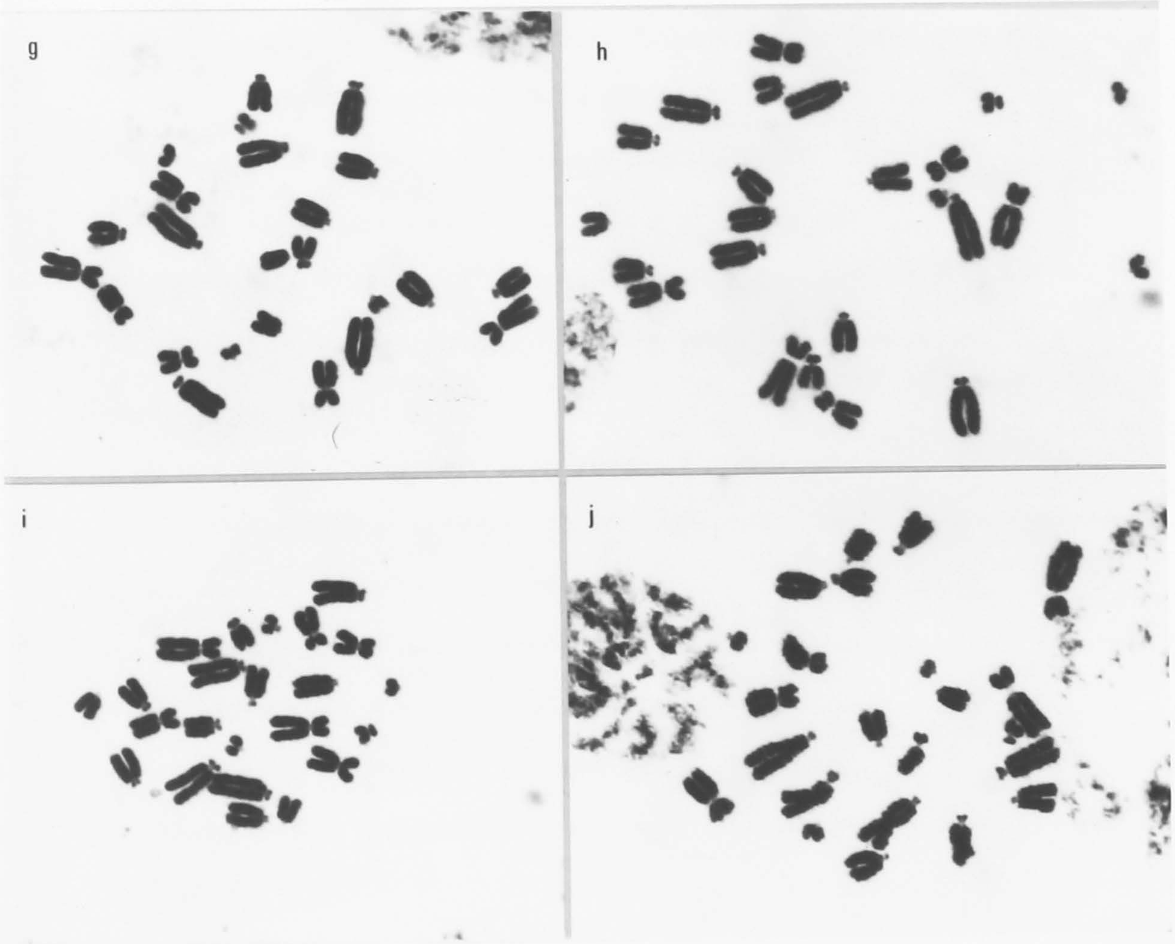
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Figure 5.4. Karyograms of laboratory reared inter-racial F1 hybrids.

(a) Note structural heterozygosity for eight pairs of chromosomes. The "Moreton" and "Torresian" forms of chromosome 9 also differ in that the "Moreton" short arm is consistently larger.

(b) This individual possesses two different acrocentric forms of chromosome 1. The "Moreton" acrocentric form has a larger short arm.

(c) Male hybrid with metacentric X chromosome.

(d) Male hybrid with "Torresian" acrocentric X chromosome.

(e) Male hybrid with "Torresian" acrocentric X chromosome. This individual also possesses two different acrocentric forms of chromosome 1.

(f) Male hybrid with "Torresian" acrocentric X chromosome.

(g) Female hybrid with two acrocentric X chromosomes, one from the "Moreton" and one from the "Torresian" race.

(h) Female hybrid same as above. Note that in both these cases the "Moreton" form of chromosome 9 is distinctly acrocentric.

(i) Male hybrid with a "Moreton" acrocentric X chromosome.

(j) Male hybrid with a "Torresian" acrocentric X chromosome.


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the hybrids. This acrocentric form of the largest autosome has a larger short arm than the "Torresian" acrocentric chromosome in the hybrids. Thus it may represent an independent polymorphic variant in the "Moreton" race and possibly may not be derived by introgression from the "Torresian" race. However until the behaviour of the "Torresian" form of chromosome 1 is investigated in F2 or backcross generations, after it has recombined with a "Moreton" homologue, the question of whether this represents a primary or secondary variant cannot be resolved. A similar variant was found in a C-banded hybrid preparation (Fig. 5.2) and clearly warrants further investigation.

5) *Quantitative analysis of meiotic anomalies in the laboratory raised hybrids*

a) *The frequency of asynapsis and other anomalies*

Asynapsis has been reported in the meiosis of hybrids of grasshoppers (Helwig, 1955; White, 1957; John and Lewis, 1965; White *et al.*, 1969; Mrongovius, 1975; John and Weissman, 1977), newts (White, 1946; Callan and Spurway, 1951; Lantz and Callan, 1954) and stick insects of the genus *Didymuria* (Craddock, 1971). It is also found in interspecific hybrids of *Drosophila* (Dobzhansky, 1970). However the frequency of asynapsis is highly variable even within the hybrids derived from a particular cross (Helwig, 1955) and in the case of the *Chorthippus* hybrids (Klingstedt, 1939), univalents were not observed at all.

The biological significance of asynapsis lies in the possible reduction in the fertility of the hybrids which may result from it. This reduction in fertility can occur in two ways. First, there may be random segregation of univalents at anaphase 1. In this case, at least 50% of the gametes produced will be aneuploid, depending on the number of asynapsed pairs, and these will decrease the fertility of the hybrids. Alternatively, the univalents may lag at anaphase 1 and block cytokinesis. Polyploid, rather than aneuploid, gametes will then be produced.

Figure 5.5. Asynapsis in inter-racial F1 hybrids.

(a) Two chromosome 10 univalents in ("Torresian" x "Moreton" metacentric X) F1 hybrid.

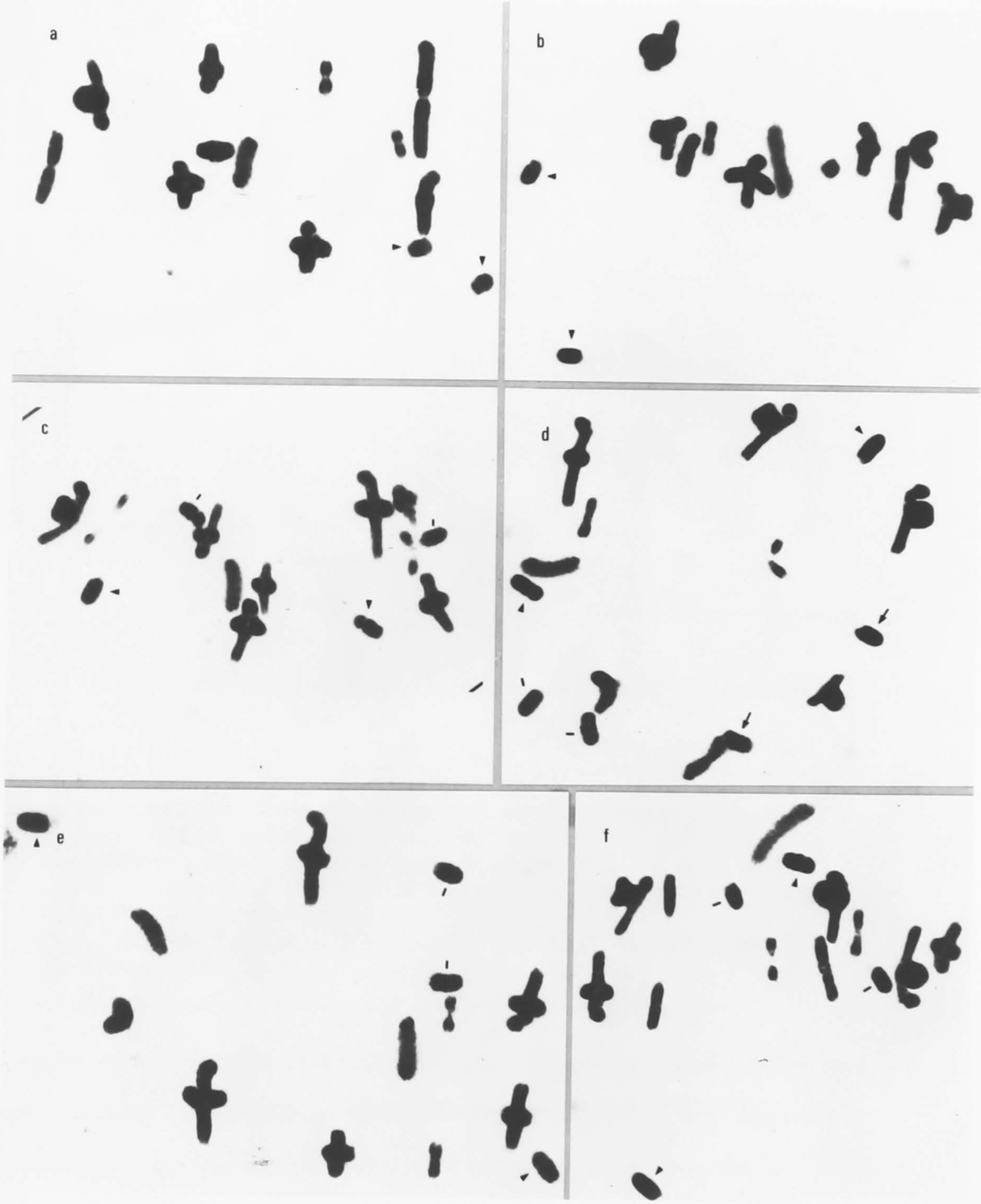
(b) Two chromosome 10 univalents in ("Moreton" metacentric X x "Torresian") F1 hybrid.

(c) Four univalents in ("Torresian" x "Moreton" metacentric X) F1 hybrid. The two clearly heteromorphic asynapsed pairs are chromosomes 5 and 6.

(d) Six univalents in a cell from ("Moreton" acrocentric X x "Torresian") F1 hybrid. One of the univalents is associated with the pair 10 bivalent. Pairs 5, 6 and 8 are asynapsed.

(e) Four univalents in ("Torresian" x "Moreton" metacentric X) F1 hybrid. The univalent pairs are chromosomes 5 and 6.

(f) Four univalents in ("Moreton" metacentric X x "Torresian") F1 hybrid. Pairs 5 and 10 are asynapsed. Pair 7 has precociously separated in this cell but is still connected by a thin strand of chromatin, which is not visible in this photograph.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The frequencies of meiotic anomalies in the laboratory raised hybrids can be compared with the control values presented in Table 5.4.

TABLE 5.4

	Univs								
	0	2	4	6	8	Multiv.	Tetra	Anom.	Total
Torresian	113	2							115
(3)	98.3	1.7							%
Moreton acro X	251	1	1					1	254
(3)	98.8	0.4	0.4					0.4	%
M x A	448	7							455
(6)	98.5	1.5							%
A x M	330	10							340
(6)	97.1	2.9							%
M x T	332	81	10	1		31	2		447
(6)	72.0	18.1	2.2	0.2		6.9	0.5		%
T x M	393	97	21	5	1	21			538
(6)	73.1	18.0	3.9	0.9	0.2	3.9			%
A x T	85	21	3			11	5	12	137
(2)	62.0	15.3	2.2			8.0	3.7	8.8	%
T x A	195	82	11	2		32			322
(3)	60.6	25.5	3.4	0.6		9.9			%

Quantitative analysis of anomalies at metaphase 1 of meiosis in lab. reared inter- and intra-racial hybrids and controls. The Moreton meta X control was not examined, but see Table 5.1 for an estimate of the frequency of anomalies in field collected individuals of this type.
(M = "Moreton" metacentric X; A = "Moreton" acrocentric X; T = "Torresian")

It can be seen that the percentage of cells showing no anomalies at metaphase 1 ranges from 98.3% to 98.8% in the "Torresian" and "Moreton" acrocentric X controls respectively. Unfortunately, because of the high mortality in the "Moreton" metacentric X control, no laboratory reared males of this type have been analysed. However, the "Moreton" intra-racial reciprocal hybrids show a similar range of 97.1% to 98.5%. The inter-racial hybrids, on the other hand, have a considerably higher frequency of anomalous cells. The percentage of cells with no anomalies ranges from 60.6% to 73.1%. The average frequency of asynapsis and the observed range in the controls and hybrids are presented in Table 5.5. There is very little overlap between the hybrids and controls. In fact, the average frequency of asynapsis in the inter-racial hybrids is about 20 times higher than the frequency in the parental controls. The differences in the frequency of asynapsis between the controls and the inter-racial hybrids are very highly significant (Table 5.6). However none of the differences between the controls and intra-racial hybrids are significant.

TABLE 5.5 Frequency of asynapsed bivalents - laboratory reared individuals

	Mean %	Range %	Homogeneity
"Torresian" control	1.74	0 - 20*	2.48(1) N.S.
"Moreton" acro X control	1.19	0 - 3.80	–
M x A	1.54	0 - 4.00	5.29(4) N.S.
A x M	2.94	0 - 8.22	9.69(4) *
M x T	25.12	4.54 - 32.58	12.71(9) N.S.
T x M	30.56	10.29 - 90.00	153.32(10) ***
A x T	24.77	24.29 - 25.64	1.72(2) N.S.
T x A	37.24	33.33 - 41.00	1.37(4) N.S.

Mean frequency of asynapsed bivalents in lab. reared hybrids and controls. All other anomalies have been excluded. The frequency is calculated from pooled data, some of which are heterogeneous. The high upper range limit for the "Torresian" control is the result of one individual for which only ten cells could be scored, 2 of which contained univalents.

The frequency of asynapsis differs significantly between some of the inter-racial crosses (Table 5.6). However the hybrids derived from "Torresian" females and "Moreton" metacentric X males have a highly heterogeneous frequency of asynapsis (Table 5.5), so that the statistical comparisons may not be valid.

TABLE 5.6

	A	M	MxA	AxM	MxT	TxM	AxT	TxA
T	0.39 N.S.	0.46 N.S.	0.15 N.S.	0.78 N.S.	8.49 ***	10.66 ***	4.68 ***	9.47 ***
A	.	1.26	0.39 N.S.	1.52 N.S.	9.36 ***	11.71 ***	4.90 ***	9.98 ***
M		.	0.96 N.S.	0.40 N.S.	8.92 ***	11.28 ***	4.66 ***	9.67 ***
MxA			.	1.28 N.S.	9.31 ***	11.69 ***	4.84 ***	9.93 ***
AxM				.	8.42 ***	10.70 ***	4.50 ***	9.36 ***
MxT					.	1.67 N.S.	0.06 N.S.	2.93 **
TxM						.	1.14 N.S.	1.60 N.S.
AxT							.	2.20 *

Significance of difference in frequency of asynapsis based on a normal test of the difference of the means of two Poisson distributions (Bailey, 1959). The heterogeneity of the AxM and TxM data have been ignored in this test. The "Moreton" metacentric X comparisons have been based on the frequency of asynapsis in field caught individuals, although these data are heterogeneous. * 5%, ** 1%, *** 0.1%

b) The distribution of asynapsis among chromosome pairs

In the controls and intra-racial crosses, the very low frequency of univalency has precluded a statistical comparison of the chromosomal distribution of asynapsis. In the "Torresian" control, only the chromosome 10 pair was ever found asynapsed. Pairs 6, 7 and 10 were occasionally observed as univalents in the "Moreton" acrocentric X control and pairs 2, 4, 9, 10 and 12 were found asynapsed in rare cells in the "Moreton" intra-racial hybrids. No chromosomes appeared to show a greater tendency for asynapsis than any others in the controls, although chromosome 10 was found asynapsed at a very low frequency in some individuals from all controls and intra-racial crosses.

The frequency of asynapsis in the inter-racial F1 crosses, on the other hand, is sufficiently high to allow a statistical comparison of the pattern of asynapsis between them, although the pattern of inter-individual variability could not be assessed. Of the total of 412 asynapsed pairs observed in the four classes of hybrids, 406 have been identified (Table 5.7). There are two possible sources of misidentification due to the similarities between chromosomes 1 and 2 and chromosomes 7 and 8. However, asynapsis of the 1 and 2 pairs was so infrequent that in practice, misidentification posed no problem. Pairs 7 and 8 on the other hand contributed 11.6% of the observed univalents, but since the possible misidentification is restricted to these pairs, and not to chromosome 6 or 9 as well, misidentification is unlikely to be a serious problem.

The distribution data from each of the four hybrid classes (Table 5.7) have been tested for heterogeneity. The contingency chi square value of 27.04 with 27 degrees of freedom is not significant and it is therefore valid to pool the data from the four reciprocal crosses. Thus although there are differences in the overall frequency of asynapsis (Table 5.6) between the crosses, they are not attributable to differences in the distribution of asynapsis among chromosome pairs.

TABLE 5.7

	Chromosome												
Cross	1	2	4	5	6	7	8	9	10	11	12	?	Total
MxT	1	4	11	16	25	5	3	8	21	12	6	1	113
TxM	2	0	7	26	32	16	7	14	25	15	9	3	153
AxT	0	0	2	3	4	3	2	2	6	0	2	-	24
TxA	1	2	10	26	26	8	3	13	11	14	3	2	117
Pooled	4	6	30	71	87	32	15	37	63	41	20	6	412

The distribution of asynapsis among chromosome pairs in the four classes of inter-racial hybrids

TABLE 5.8

	Analysis by		
Cross	Chromosome (10 d.f.)	Chromosome size class (3 d.f.)	Sample size
MxT	58.70 (***)	23.41(***)	112
TxM	75.99(***)	44.40(***)	153
AxT	15.42(N.S.)	11.98(**)	24
TxA	73.38(***)	31.98(***)	117

Analysis of asynapsis by chromosome and by chromosome size class on the null hypothesis of a uniform distribution of asynapsis. ** 1% *** 0.5% N.S. Not Significant

Chromosome size classes	L	M	S-M	S
	1,2	4,5,6,7,8	9,10	11,12

The pooled distribution data are displayed in the form of a histogram in Figure 5.6 and clearly show a biomodal distribution with peaks of asynapsis for pairs 6 and 10. An analysis of the data for each of the hybrid crosses has revealed that the distribution of asynapsis is not uniform (Table 5.8). The asynapsis observed in the hybrids may be related to the distal localization of chiasmata also seen in them. However, this does not provide an explanation for the non-uniform distribution of asynapsis among chromosome pairs.

For both naturally occurring and induced asynapsis, the distribution of univalency among chromosome pairs has been examined in most detail in grasshoppers. Patterns of asynapsis, varying from univalency which affects the large chromosomes but not the smalls (Rees, 1957) to asynapsis which affects only the four smallest chromosomes but has no effect on the pairing of the large chromosomes (John and Naylor, 1961), have been reported in naturally occurring asynaptic mutants. Heat treatment progressively affects synapsis of the small chromosomes, then the long chromosomes and finally the medium sized chromosomes (Henderson, 1963). Furthermore, a desynaptic mutant has been found in the plant species, *Hypochieris radicata*, in which synapsis fails in one specific chromosome pair, with normal pairing of the other chromosomes (Parker, 1975). Thus there is no consistency in the pattern of univalency in the mutants or experimentally induced cases of asynapsis.

The pairing of homeologous chromosomes in hybrids can be disrupted by two possible mechanisms. First, structural rearrangements, whether cryptic or visible, can prevent normal synapsis of the homologues. In this case, there should be some correlation between the frequency of asynapsis in particular chromosomes and the presence of visible rearrangement differences in the same chromosome. Further, the frequency of asynapsis should be consistent among individuals heterozygous for the same rearrangements. Secondly, hybrid genotypic imbalance may also

Figure 5.6. Distribution of asynapsis among chromosomal pairs in laboratory raised inter-racial F1 hybrids. The distribution is based on the pooled data from the four types of reciprocal inter-racial hybrids.

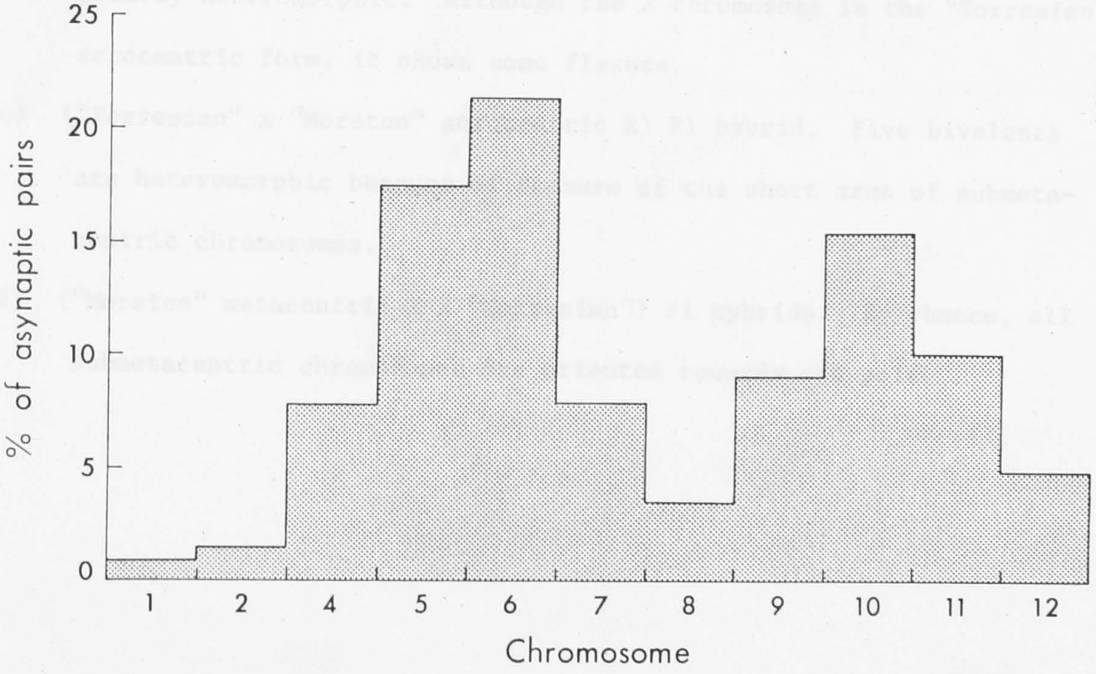

25
20
15
10
5
0
% of asynaptic pairs
1
2
4
5
6
7
8
9
10
11
12
Chromosome

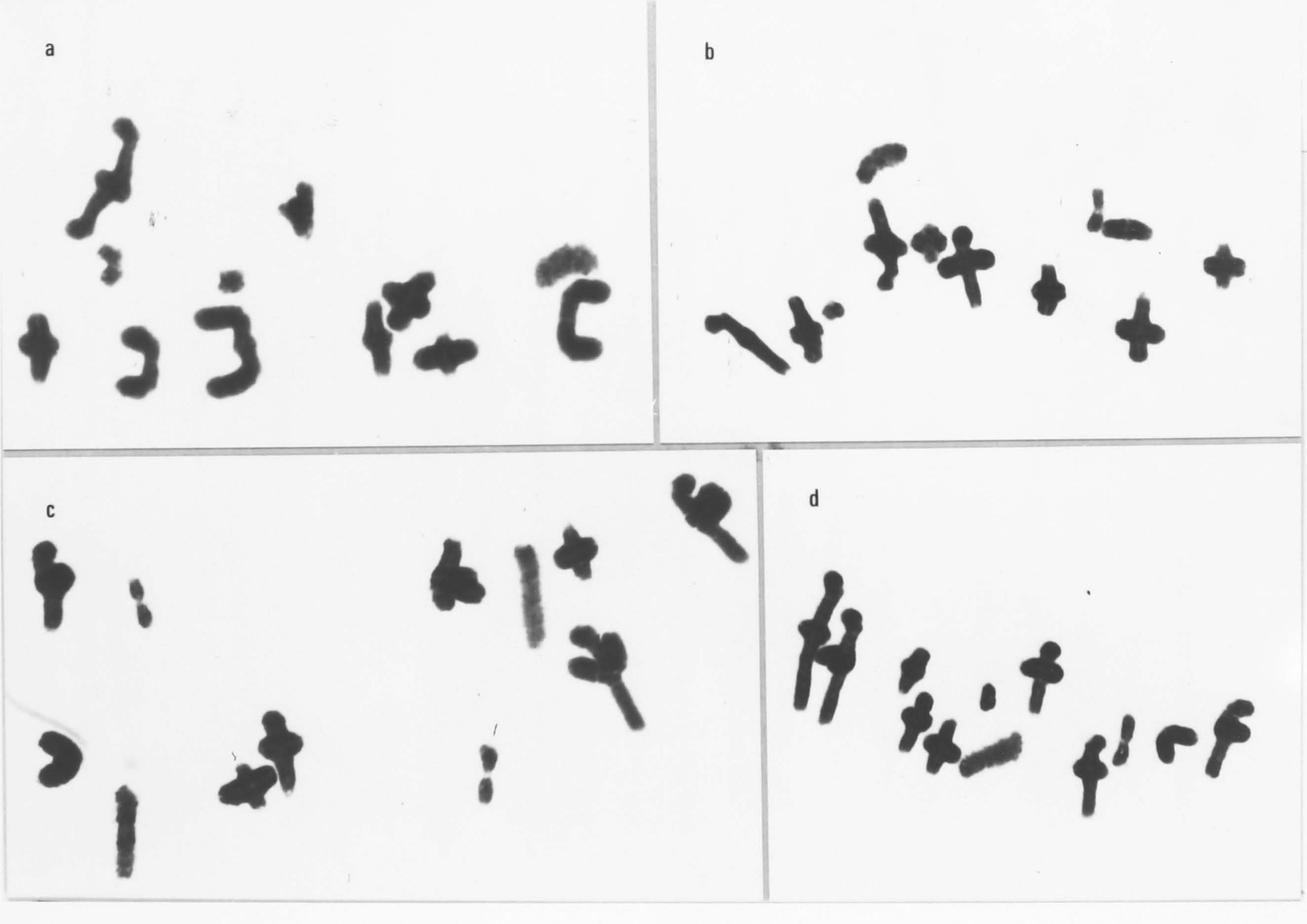
Figure 5.7. Normal metaphase 1 cells in inter- and intra-racial F1 hybrids.

(a) ("Moreton" metacentric X x "Moreton" acrocentric X) intra-racial F1 hybrid. Note structurally homomorphic bivalents and flexure of the metacentric X chromosome.

(b) ("Torresian" x "Moreton" metacentric X) F1 hybrid. Six bivalents are clearly heteromorphic. Although the X chromosome is the "Torresian" acrocentric form, it shows some flexure.

(c) ("Torresian" x "Moreton" acrocentric X) F1 hybrid. Five bivalents are heteromorphic because of flexure of the short arms of submetacentric chromosomes.

(d) ("Moreton" metacentric X x "Torresian") F1 hybrids. By chance, all submetacentric chromosomes are oriented towards one pole.


a
b
c
d

* Given that the submetacentric chromosomes are derived from primitive acrocentric/telocentric elements and given the observed metaphase 1 configurations in chromosomal heterozygotes, it is valid to use the size of the centromere shift as an indicator of the size of the pericentric inversion.

cause asynapsis. However, in this case, the frequency and distribution of asynapsis among hybrid individuals would not necessarily be homogeneous. Similarly there need be no correlation between the distribution of asynapsis and the presence of chromosomal rearrangements.

The 37 hybrids between *Circotettix verruculatus* and *Trimerotropis suffusa* examined by Helwig (1955) were highly heterogeneous both in the frequency and distribution of asynapsis among chromosome pairs. Because of this inter-individual variation and the almost total lack of asynapsis in the parental species, Helwig concluded that it was very unlikely that cryptic rearrangements have played any role in causing the asynapsis observed in these hybrids. Nevertheless this pattern of asynapsis bears some resemblances to the distribution of univalents seen in the hybrids between the "Moreton" and "Torresian" races. In particular, the large chromosomes in both the Trimerotropine and *Caledia* hybrids are very rarely asynpased. This similarity exists despite the obvious rearrangement differences in the large chromosomes of the *Caledia* hybrids and the absence of such differences in the Trimerotropine hybrids. This is evidence that the rearrangements themselves are not causing failure of pairing in the *Caledia* hybrids.

Likewise a comparison of the frequency of asynapsis between chromosomes in the *Caledia* hybrids provides evidence that the rearrangements are not causing asynapsis. For example, chromosome 1, which has a* large rearrangement difference in the hybrids, contributed only 0.98% of the observed univalent pairs. On the other hand, chromosome 9, which has only a very* small difference in the position of the centromere, contributed 9.11% of the asynapsed pairs. Chromosome 6, which has a rearrangement difference approximately equivalent to that of chromosome 1, produced 21.43% of the univalents found in the hybrids. All of these cases provide support for the role of hybrid genotypic imbalance, rather than structural rearrangements, in causing asynapsis. Furthermore, in non-hybrid members of the "Moreton" race, which were structurally heterozygous for one or two identified chromosome pairs, there was no asynapsis in either the heteromorphic pair or indeed the rest of the complement (Section 2). Again this demonstrates that the structural rearrangements *per se* do not cause the observed asynapsis.

c) Other anomalies at metaphase 1

Three other categories of meiotic anomalies have been observed and recorded during the quantitative analysis of metaphase 1 cells. The first category is a heterogeneous class of anomalies, ranging from almost complete asynapsis to the presence of fragments, multivalents and univalents. Almost all of these anomalous cells were observed in one hybrid individual derived from the cross between "Moreton" acrocentric X females and "Torresian" males and occurred in a single cyst of metaphase 1 cells. A single anomalous cell containing many univalents and fragments was also found in the "Moreton" acrocentric X control. It is unlikely that these anomalies are related to hybridity.

Tetraploid metaphase 1 cells were observed in hybrids derived from crosses between both "Moreton" metacentric X and acrocentric X females and "Torresian" males (Table 5.4). They have also been observed in the "Moreton" metacentric X field control in one individual (Table 5.1). The frequency of occurrence of such cells is low and they are unlikely to have a signficant effect on the fertility of the hybrids. In hybrid tetraploid cells, pairing was generally homeologous, although there was some multivalent formation and possibly homologous pairing. This contrasts with the situation in hybrids between the "Daintree" and "Moreton" races (Shaw, pers. comm.), where there is a high frequency of formation of tetraploid meiotic cells, in which there was regular homologous pairing. The tetraploid metaphase 1 cells are presumed to be the result of failure of cytokinesis at the final premeiotic mitosis.

The presence of multivalents in some F1 hybrids was the frequent anomaly other than univalents. These were found in all classes of F1 inter-racial hybrids. Multivalents were not found either in the controls or the progeny of intra-racial crosses. They were however present in some F1 hybrids collected in the field (Table 5.1). Multivalents were found in 4-10% of the cells in the four classes of laboratory reared hybrids (Table 5.4). The multiples were generally quadrivalents, although occasional trivalents and univalents were observed. Multiple formation in F1 hybrids is usually interpreted as evidence for fixed translocation differences between the parental types involved in the hybridization. However, in this case, the nature of the chromosomal differences between the "Moreton" and "Torresian" races makes it possible to identify the chromosomes involved in the associations at metaphase 1, when the short arms of the submetacentric chromosomes are flexed (Fig. 5.8). It can be seen that the majority of the multiples are the result of associations between non-homologous members of the same parental gametic complement (Table 5.9). Of the 88 multiple associations observed in the hybrids, 53 were between the "Torresian" ends of two bivalents, 9 were between the "Moreton" ends of two bivalents and only 2 were between a "Torresian" and a "Moreton" end of two bivalents. If these represented translocation configurations, all multiples would fall into this final category. A further 24 associations could not be categorized because of the occurrence of acrocentric-telocentric chromosomal morphs in the "Moreton" complement for chromosomes 7, 8, 9 and 10 (Table 5.9). Nevertheless it is clear that the multiples are not the result of translocation differences even in these cases, because there is not a consistent pattern of multiple formation.

The association of non-homologous chromosomes in these hybrids is quite variable. Of the total of 55 possible combinations of non-homologous pairs involved in the multivalents, 26 have been observed (Table 5.10). The most commonly observed multiple involved pairs 5 and 6. The combination

Figure 5.8. Multivalent formation in inter-racial F1 hybrids.

(a) Association between the "Torresian" ends of two large bivalents in a ("Torresian" x "Moreton" acrocentric X) F1 hybrid. The multiple is disjunctionally oriented.

(b) Association between the "Torresian" end of a large bivalent and a bivalent consisting of two acrocentric chromosomes. In this case, the multivalent is linearly oriented. The individual is a ("Torresian" x "Moreton" acrocentric X) F1 hybrid.

(c) A rare association involving the "Moreton" end of the number 2 bivalent and the "Torresian" end of the number 4 bivalent in a ("Moreton" metacentric X x "Torresian") F1 hybrid. Chromosome 12 is also asynapsed in this cell.

(d) A "Moreton"-"Moreton" association in a ("Moreton" metacentric X x "Torresian") F1 hybrid. The multivalent is disjunctionally oriented. Four univalents are also present in this cell.

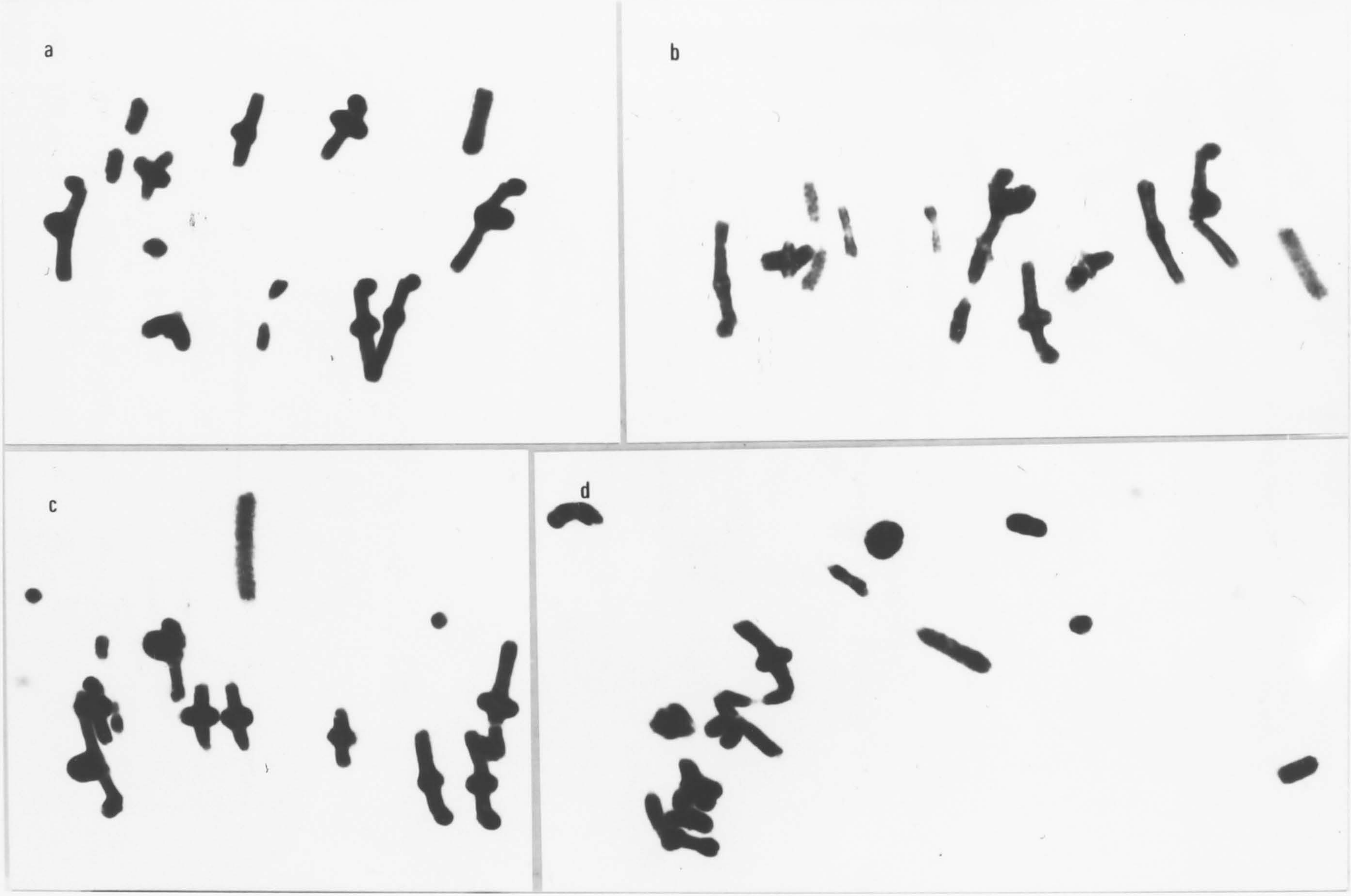

a
b
c
d

TABLE 5.9

	Number of associations			
Cross	tt	tm	mm	Not classified
MxT	13	1	3	11
TxM	14	0	3	3
AxT	4	0	0	5
TxA	22	1	3	5
Total	53	2	9	24

The number of chromosomal associations and the chromosome ends involved in maintaining the multiples in the inter-racial hybrids. t - "Torresian", m - "Moreton".

TABLE 5.10

Chromosome pairs involved in multivalents

	1	2	4	5	6	7	8	9	10	11
2	10									
4	4	3								
5	2	2	9							
6	3	4	10	16						
7	2	2	2	1	1					
8	1	0	0	0	0	0				
9	2	0	0	1	0	1	1			
10	0	0	2	1	0	1	1	1		
11	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	1

of pairs 1 with 2, 4 with 5 and 4 with 6 were also relatively common. On the other hand, chromosomes 11 and 12 were involved in only one association out of 21 possibilities. The identified "Moreton"-"Torresian" associations were between chromosomes 1 and 4 and chromosomes 2 and 5. However it is not obvious why the associations should be mainly between non-homologous "Torresian" chromosomes or why some pairs should be involved more frequently than others.

The nature of the associations between non-homologous chromosomes in the multiples is not clear. All of the observed associations were, however, terminal and persistent (Fig. 5.8). Further the majority of the multiples were disjunctionally oriented. Linearly oriented multiples were rarely found in cases where there was a large difference in the size of the non-homologous pairs (Fig. 5.8). The associations are therefore unlikely to cause the formation of unbalanced gametes, although they may cause a slight distortion of segregation.

Similar associations, some of which were originally interpreted as evidence for translocation heterozygosity, have been reported in hybrids between species of grasshoppers (Helwig, 1955; White, 1957b; John and Lewis, 1965), newts (Callan and Spurway, 1951; Lantz and Callan, 1954) and stick insects (Craddock, 1971). They have also been reported in natural populations of grasshoppers (White, 1961; Nankivell, 1967). In the diploid meiosis of hybrids between the "Moreton" and "Daintree" races of *Caledia captiva*, non-homolgous associations affecting many chromosomes are found in all cells (Shaw, pers. comm.). In this case also, most of the associations are between non-homologous members of the same parental gametic complement and clearly cannot be the result of translocation differences.

Since the occurrence of multiples is restricted to hybrids, both in the "Moreton"-"Torresian" and the "Moreton"-"Daintree" cases, hybrid genotypic imbalance must be implicated in these meiotic disturbances.

White (1973) considers that such situations may result from duplication and translocation of minute terminal regions between chromosomes, so that there are small regions of homology. When the normal synaptic pattern is disrupted, for example in hybrids, synapsis and chiasma formation can occur in these regions. However this hypothesis implies greater homology among the "Torresian" ends of chromosomes than among the "Moreton" ends of non-homologous chromosomes.

6) Crossing over within a pericentric inversion

It has long been established that straight pairing occurs in grasshopper chromosomes which are heterozygous for centric rearrangements (Coleman, 1948; White and Morley, 1955). On the assumption that the rearrangements are pericentric inversions, the straight pairing will be non-homologous in inversion heterozygotes and will thus prevent the formation of chiasmata in the inverted segments. Consequently there will be no reduction in fertility because of the production of duplication-deficiency crossover products. Straight pairing can be observed in the early stages of meiosis of hybrids between the "Moreton" and "Torresian" races and also in structurally heterozygous members of the "Moreton" race.

However several meiotic cells have been observed in the inter-racial hybrids which contain configurations which could result from crossing over in the inverted region of the chromosome. The crossover products (Fig. 5.9) require homologous pairing of the short arm of the submetacentric chromosome with the equivalent segment in the acrocentric chromosome, either with or without the formation of an inversion loop. Crossing over in the non-inverted segment of course does not affect the results of the chiasma in the inverted segment. In an F1 hybrid male derived from the cross between "Torresian" females and "Moreton" acrocentric X males, two anaphase 1 restitution nuclei were observed with duplication-deficiency products which could result from such a

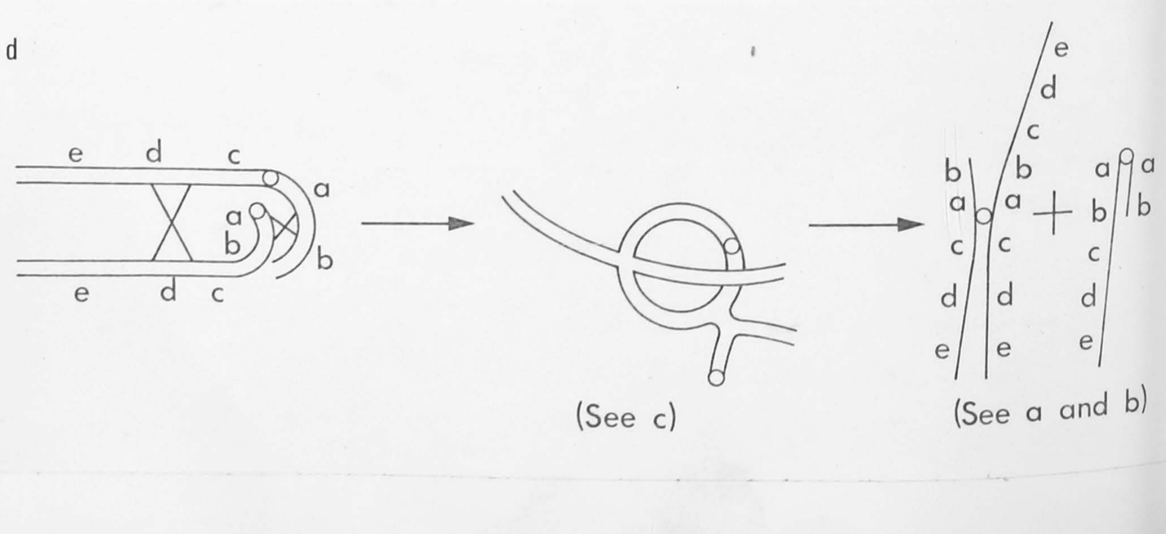
Figure 5.9. Evidence for rare crossing over in pericentric inversions.

(a) Anaphase 1 restitution nucleus containing duplication (du) and deficiency (de) products which would result from crossing over in a heterozygous pericentric inversion. The individual is a ("Torresian" x "Moreton" acrocentric X) F1 hybrid.

(b) Another restitution nucleus in the same individual as above. The duplication and deficiency products are indicated and in this case a fragment (arrowhead) is also present. The other anomalous bivalent in this cell has not been interpreted.

(c) A metaphase 1 nucleus in a ("Moreton" metacentric X x "Torresian") F1 hybrid, containing a bivalent (arrowed) with a configuration apparently resulting from reverse loop pairing and crossing over in the pericentric inversion.

(d) Diagramatic representation of crossing over in a pericentric inversion heterozygote. Although two crossovers are drawn in this diagram, it is possible that there is only one crossover (in the inverted region) in the bivalent arrowed in 5.9c. The metaphase I bivalent configuration resulting from a second crossover (in the non-inverted region) close to the position of the centromere of the submetacentric chromosome is difficult to distinguish cytologically from a single crossover bivalent.


d
e
d
c
a
a
b
b
e
d
c
(See c)
e
d
c
b
b
a
a
c
c
d
d
e
e
a
a
b
b
c
d
e
(See a and b)


a
du
de
b
de
du
c

crossover. In one of these cases, a fragment has also been produced, presumably because of failure of rejoining to the deficient arm of the acrocentric chromosome. A metaphase 1 cell was also recorded in a "Moreton" metacentric X female cross "Torresian" male hybrid with a large bivalent showing what has been interpreted as a reverse loop configuration (Fig. 5.9). In this case, there has been a crossover in both the inverted and non-inverted segments.

Such cells were very rare and were recorded only because of their unusual appearance. The majority of bivalents in metaphase 1 cells had only one chiasma in a terminal position on the long arm, where the crossover event could not cause any meiotic difficulties. Further the majority of anaphase 1 cells observed were normal, although failure of cytokinesis was not uncommon. However anaphase 1 cells were not found frequently enough to permit a quantitative assessment of anomalies at this stage of meiosis. Although crossing over within a pericentric inversion poses no barrier to cell division at anaphase 1, both observed examples of inversion crossover products were found in anaphase 1 restitution nuclei. If the two events are related, the already small effect of these rare crossover events will be even further reduced.

7) Restitution nuclei and polyploid spermatids

The failure of cytokinesis at either first or second anaphase will produce gametes with a diploid rather than a haploid complement of chromosomes. The failure of both of these divisions will give rise to tetraploid gametes. If a tetraploid cell enters meiosis and cytokinesis does not occur at both anaphase 1 and anaphase 2, an octoploid gamete will be produced. In the inter-racial hybrids, diploid and tetraploid spermatids were frequently observed (Fig. 5.10). Octoploid spermatids were seen rarely, but the tetraploid metaphase 1 cells which would give rise to them were also uncommon (Table 5.4).

The restitution nuclei, resulting from failure of cytokinesis at

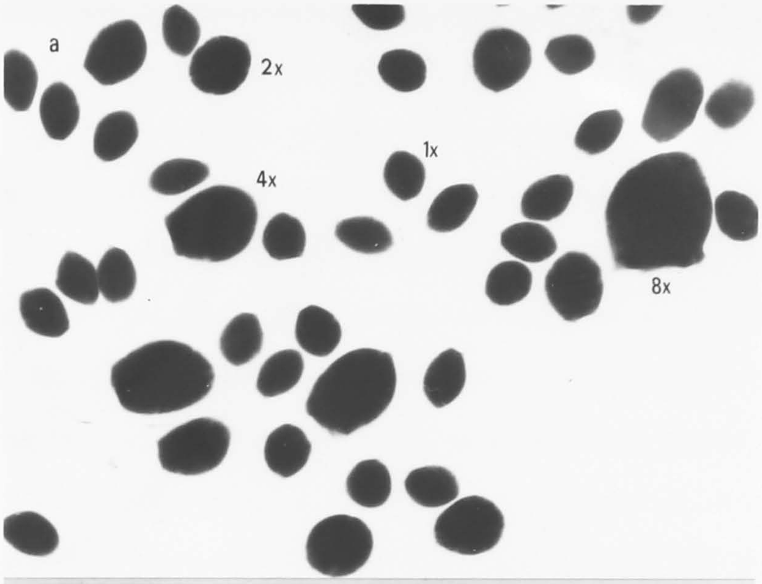
Figure 5.10. Polyploid spermatids

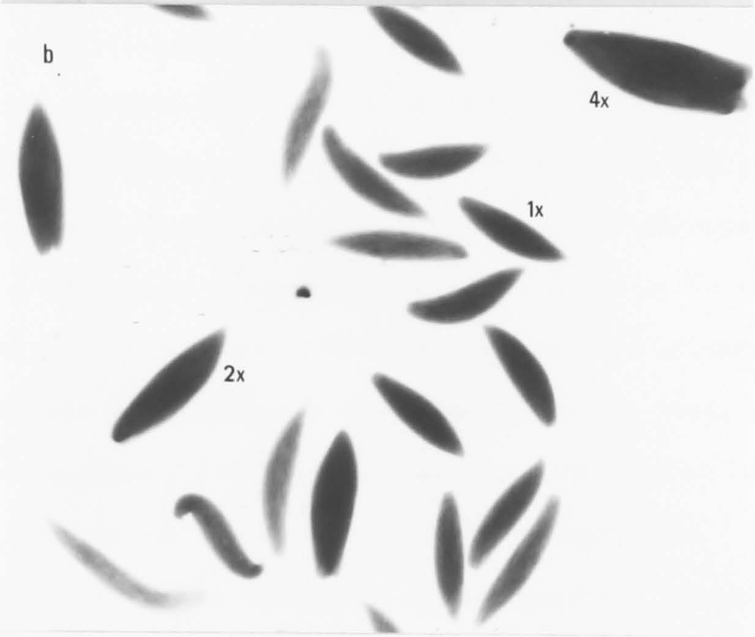
(a) Normal, diploid, tetraploid and octoploid spermatids in a ("Moreton" acrocentric X x "Torresian") F1 hybrid male.

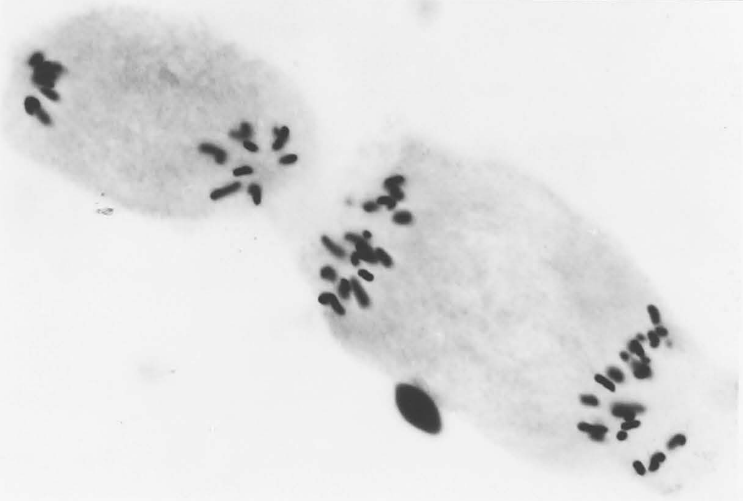
(b) Polyploid spermatids showing a greater degree of elongation in a ("Moreton" metacentric X x "Torresian") F1 hybrid.

Figure 5.11. Restitution nucleus

A normal and a diploid anaphase II cell from a cyst of anaphase II cells. There are 11 or 12 chromosomes at each pole in the normal cell, but 23 chromosomes at each pole in the cell on the right because of the failure of the reductional division. Anaphase 1 restitution nuclei are illustrated in Figure 5.9.


a
2x
1x
4x
8x


b
4x
1x
2x



anaphse 1 or anaphase 2, which eventually form these types of aberrant gametes, have also been observed (Fig. 5.11, see also Fig. 5.9). Two factors have been implicated in the failure of the first division and hence the formation of nuclei of this sort. The first and most important factor was the presence of univalents which lag on the equatorial plate, rather than being incorporated into one of the poles. The second, and in this case, less important factor was the formation of mechanical connections or bridges which prevented bivalents from separating (Fig. 5.12). However, bridges of this type were very rare. Both of these factors have also caused the formation of aneuploid rather than polyploid nuclei (Fig. 5.13), either by random incorporation of univalents into the anaphase 1 poles or by the incorporation of a bivalent joined by a bridge into one pole.

It has been well established in grasshoppers that asynapsed chromosomes and mechanical bridges can cause a failure of cytokinesis. Experimental studies (Henderson, 1962) and observations of naturally occurring asynapsis and univalency (John and Henderson, 1962; Nur, 1969; John and Weissman, 1977) have demonstrated the role of lagging univalents in causing the formation of polyploid spermatids. Similarly a study of radiation induced structural heterozygosity, for both inversions and translocations, in an unidentified species of *Aelopus*, has shown that dicentric bridges impair cytokinesis and result in the production of diploid and tetraploid sperm (Ray-Chaudhuri and Sharma, 1962). Dicentric bridges were very rare in hybrids between the "Moreton" and "Torresian" races and are considered to result more probably from U-type exchanges rather than cryptic structural heterozygosity for paracentric inversions.

Polyploid spermatids are important because they represent a gametic elimination system, which will prevent the formation of aneuploid gametes and their function in fertilization. This is not unique to grasshoppers. In *Mantis religiosa* (Callan and Jacobs, 1957),

Figure 5.12. Lagging behaviour of univalents.

(a) A single lagging univalent at anaphase 1 in a ("Torresian" x "Moreton" metacentric X) Fl hybrid.

(b) Two homologous lagging univalents at anaphase 1 in a ("Torresian" x "Moreton" metacentric X) Fl hybrid.

(c) Two lagging univalents at late anaphase-early telophase 1 in a ("Torresian" x "Moreton" metacentric X) Fl hybrid. This cell indicates that univalents are not necessarily randomly incorporated into the anaphase 1 poles. It is likely that such univalents block cytokinesis, since micronuclei are not observed, whereas polyploid spermatids are common.


a
b
c

Figure 5.13. Irregular segregation at anaphase 1

(a) Irregular anaphase 1 segregation in a ("Moreton" acrocentric X x "Torresian") F1 hybrid. There are 13 chromosomes in the pole on the left of the figure and only 10 chromosomes in the opposite pole. This is presumably the result of random incorporation of two univalents into one pole.

(b) Aneuploid anaphase 1 poles resulting from the failure of a dicentric bridge to break. Both homologous chromosomes involved in the dicentric bridge have been incorporated into the pole in the left of the figure. The dicentric bridge and fragment (arrowed) are presumed to be the result of a U-type exchange, although they could conceivably also result from crossing over in a cryptic paracentric inversion. The individual is a ("Torresian" x "Moreton" metacentric X) F1 hybrid.

a

b

it has been found that univalents block division at metaphase 1, and in conjunction with maloriented trivalents, which persist until second telophase, prevent the formation of aneuploid gametes. In the case of the mantids and grasshoppers, including the *Caledia* hybrids, the diploid and tetraploid sperm are very unlikely to function in fertilization. Thus although they represent a loss in the total number of functional gametes, they ensure that those gametes which do function in fertilization do not suffer from duplications or deficiencies. The result is a reduction in the concentration of functional sperm, but unless the level of meiotic anomalies causing polyploidization is very high, there will be no appreciable reduction in the fertility of the males. The efficiency of such a gametic elimination system has been experimentally demonstrated. Shaw (pers. comm.) has found that chromosomally deficient or duplicated progeny are not produced when heated treated *Schistocerca gregaria* males are crossed to normal females, despite the high level of asynapsis in these males. Of course, this type of gametic elimination system depends on the very high level of production of redundant sperm in males and thus could not function effectively in the female hybrids, where the total number of gametes produced is much smaller. The efficiency of the gametic elimination system in the "Moreton"-"Torresian" hybrids is difficult to estimate, since it is not known what proportion of the univalent containing cells fail to divide at the first or second division of meiosis. However, the potential effect of asynapsis on the fertility of these hybrids is likely to be considerably reduced by a polyploid gametic elimination system.

DISCUSSION

As expected, the mitotic examination of the racial hybrids has verified the differences in chromosome structure and banding pattern, which had been inferred from previous chromosomal examination of the pure "Moreton" and "Torresian" individuals. However the meiotic examination of both the field collected and laboratory reared hybrids and controls has proved far more useful than the simple observation of hybrid mitosis, because it has allowed an indirect assessment of the fertility of the F1 hybrids. Although the assessment of infertility is based on the frequency of meiotic anomalies, it is possible to distinguish between the agents causing the anomalies, namely structural rearrangements or hybrid genotypic imbalance. This allows a distinction to be made between chromosomal or genic sterility (Dobzhansky, 1970). More importantly in this case, the effects of F1 infertility can be distinguished from hybrid breakdown in the subsequent generation. The maximum upper limit of infertility in the hybrids, which could be inferred from the frequency of meiotic anomalies, is insufficient to cause the depression of progeny production per egg pod, which was observed in the laboratory F2 generation (Chapter IV). Thus, in this case, hybrid breakdown, caused by the disruption of coadpated complexes as a result of segregation, is a more likely explanation for the depression in the production of F2 progeny.

Although the F1 hybrids were generally found to have a higher average level of meiotic anomalies than the controls, the frequency of these anomalies was often heterogeneous. In the case of the field collected hybrids in particular, the frequency of asynapsis showed an observed range of 1.27% to 36.36%, and thus overlapped considerably with the pure racial controls. Therefore, even if asynapsis does cause a depression of fertility, clearly many of the F1 hybrids from the contact zone are just as fertile as the parental types. F1 infertility, in this case, cannot play a role in maintaining a stable, narrow hybrid zone. On the other hand, the frequency of asynapsis in the laboratory reared hybrids is generally higher than that observed in the field hybrids, although even here there is some variation between individuals. The lower level of meiotic anomalies in the hybrids from the contact zone may be the result of introgression, which would reduce the effect of hybrid genotypic imbalance in subsequent hybridization. A similar difference has been found between field collected and laboratory reared hybrids in the Viatica group of Morabine grasshoppers (White *et al.* 1967) and in the *Didymuria violescens* complex (Craddock, 1971). In *Didymuria*, the F1 laboratory hybrids have a mean percentage of asynapsis of 35.60%, with a range of 3.33-77.78%. The field hybrids on the other hand, have a mean frequency of asynapsis of only 1.96%, with a range between individuals of 0-15%. The range in the frequency of malorientation of trivalents is 0-20% in the field hybrids and 0-35.85% in the laboratory hybrids. Thus hybrid infertility in this case also is unlikely to be important in maintaining the narrow contact zone.

The complete data on meiotic anomalies in field and laboratory hybrids and the control crosses of the two races of *Caledia* have been summarized in terms of Merrel's isolation estimates (Table 5.11). These are not fertility estimates for the hybrids, since a polyploid gametic elimination system will maintain the fertility of the hybrids close to the control levels in spite of the anomalies. In any case, the intra-racial isolation estimate of 0.999 shows that the "Moreton" intra-racial hybrids are effectively no different from the "Moreton" controls. The laboratory reared inter-racial hybrids, on the other hand, are clearly and significantly different from the controls. However the difference between the field and laboratory inter-racial isolation estimates are striking. The field isolation estimate of 0.918 is much closer to the intra-racial estimate than to the equivalent laboratory inter-racial estimate of 0.743.

TABLE 5.11

Comparisons of level of meiotic anomalies in inter- and intra-racial hybrids. Merrel's isolation estimate, i, based on the percentage of metaphase 1 cells with no observable anomalies. The "Moreton" meta X field control data have also been used in the (A,M) and (M,T) laboratory comparisons

Comparison	Merrel's i
M,T (field)	0.918
M,T (laboratory)	0.743
A,T	0.626
A,M	0.999

Clearly the structural rearrangements, which distinguish the "Moreton" from the "Torresian" race, are not important factors in the production of meiotic anomalies in the hybrids. In any case the fertility depression resulting from these anomalies is not likely to be great. Except for the few very rare examples of crossing over within an inverted segment in the hybrids, no infertility can be directly attributed to the rearrangement differences themselves. Since hybrid infertility, which is directly attributable to chromosomal rearrangement differences, is a necessary condition of the stasipatric mode of speciation, the possibility of a stasipatric origin for the "Moreton" and "Torresian" races can be eliminated. The hybrid breakdown observed in the F2 generation and the maintenance of the hybrid zone between these races, can be better explained by the disruption of coadapted gene complexes which evolved in isolation.

CHAPTER VI

a) *Morphometric Differentiation, Racial Affinities and Geographical Variation.*

INTRODUCTION

The four chromosomal races of *Caledia captiva* can be reliably distinguished on qualitative, non-overlapping differences in chromosomal structure. However it is not possible to reliably categorise individuals of the four races on the basis of qualitative differences in external morphology. Given the extent of the inter-racial karytopic divergence and the evolutionary implications of such chromosomal changes, it is of interest to test for any subtle morphological differences which might have accrued over the period in which the chromosomal differences were accumulating. The major interest therefore lies in gauging the extent of the obviously very low level of inter-racial morphological differentiation relative to the chromosomal differences. Given some differentiation, the morphological affinities of the races can be determined and related to affinities determined by hybridization studies and other analyses.

Since any phenotypic differences which exist between the races are obviously small and cryptic relative to the intra-racial variation, the multivariate technique of Canonical Variate Analysis or multiple discriminant function analysis has been employed. Data, which have been collected for a number of characters measured on a sample of individuals from each population are simultaneously analysed. The simultaneous analysis of several character variables will obviously extract more information than an analysis of one variable at a time since it takes into account the variation or uniformity in the intra-group covariation between characters. The usefulness of the technique lies in the fact that the variation between populations is maximised relative to the variation within each population. Further the number of dimensions of the differentiation is reduced to a level which can be easily visualised. The advantages therefore are data reduction and dimension reduction so that the interpopulation variation can be easily interpreted.

An important biological assumption of canonical analysis is that there is only one taxon per group (Thorpe, 1976). The karyotypic differences between the chromosomal races of *Caledia captiva* (Shaw, 1976; Chapter I) allow an unambiguous *a priori* identification of individuals. Further the geographical position and extent of racial sympatry between the "Moreton" and "Torresian" races in south east Queensland (Chapter III) and the "Torresian" and "Daintree" races in Cape York (see Chapter II) is sufficiently well known to exclude the possibility of sympatry in any of the populations which were not completely analysed cytologically. Thus the requirement of one taxon per group has been satisfied.

The function of Canonical analysis is to determine linear combinations of the original characters called canonical variates. These are chosen so that the variation between the population means along the canonical axes delineated by these linear combinations or discriminant functions is maximised. The first canonical variate or axis is chosen such that it is inclined in the direction of greatest variation between the population means, relative to the variation within each population. The second axis is chosen so that it is uncorrelated within populations with the first axis and so that the variation between population means relative to the within population dispersion is next greatest (Campbell, pers. comm; see also Blackith and Reyment, 1971; Cooley and Lohnes, 1971). The process is repeated until all possible cannonical axes are exhausted and all interpopulation variation is accounted for. The effect of the maximising process in determining the canonical axes is to concentrate the interpopulation variation in the first few axes, so that only a reduced number of dimensions may need to be considered. The difficulty then lies in interpreting the biological significance of the interpopulation variation expressed in this way by relating it to race, geographical location or other biologically important factors.

The discriminant functions, which are linear combinations of the character variables, assign a loading to each of the characters in each canonical axis so that the interpopulation variation is maximised as described previously. For single discriminant function analysis, in which only two populations are being compared along one axis, the absolute size of the character loading gives an estimate of the contribution of each character to the interpopulation or inter taxon discrimination. Characters with very small loadings contribute little to discrimination and can be excluded without greatly increasing the probability of misidentifying randomly chosen individuals. However canonical analysis or multiple discriminant function is more complicated since more than two populations are being compared and often more than two taxa. Hence there is a corresponding increase in the number of axes of discrimination. If the principle aim of the analysis is to distinguish between taxa for which there are several representative populations, it may be difficult to determine which canonical axis or axes reflect inter-taxon variation and which account for interpopulation variation not related to taxon. Consequently the *a posteriori* exclusion of characters based on the contribution of each character to each vector or discriminant function (Thorpe, 1976) must be carried out cautiously so that biologically relevant characters are not excluded. Nevertheless since the characters are chosen for the analysis without any *a priori* knowledge of their usefulness in discriminating between populations or taxa, it must be assumed that some characters contribute little to discrimination and can be usefully eliminated. The removal of non-discriminating characters may improve the interpopulation discrimination and will certainly increase the percentage variation accounted for by the first few roots. One possible assumption that can be made is that the inter-racial variation will be sufficiently large relative to the other interpopulation variation that it will be accounted for in the first two or three canonical variates in which most interpopulation variation is concentrated. Hence the character variables which contribute little to the first three canonical variates can be exculded using *a posteriori* statistical criteria, with the aim of improving inter-taxon discrimination by removing the non-contributing variables. If an improvement is obtained, the character elimination is biologically valid. A failure to improve discrimination between taxa will mean either that there are no differences between taxa in the given characters or that the statistical exclusion criteria have caused biologically relevant variables to be excluded. The latter possibility has been pointed out by Blackith and Reyment (1971).

In any multivariate study, it is important to obtain enough specimens from each locality to take account of local site variation and also to sample enough localities to represent the geographical variation in the taxa under consideration. The primary aim of the analysis presented here is a study of the morphological variation within and between the "Moreton" and "Torresian" races. For both races, adequate samples from representative localities throughout their geographical distributions have been examined and thus intrapopulation and interpopulation variability have been sufficiently sampled. A complementary analysis of the "Daintree" and "south east Australian" races as well as of *Caledia* species *nova 1* has also been carried out to determine the affinities of the "Moreton" and "Torresian" races to these other taxa. Only in the case of the "south east Australian" race has the interpopulation within taxon, geographical variation been sampled. The examination of the "Daintree" race and of *C.* species *nova 1* is less satisfactory since only a single locality has been sampled in each case.

The application of canonical variate analysis to morphometric problems has been frequently reported in the literature of zoology, anthropology and paleontology (for reviews see Blackith and Reyment, 1971; Atchley and Bryant, 1975; Bryant and Atchley, 1975; Thorpe, 1976). Of particular relevance to this study are the applications of canonical variate analysis to grasshopper species, since it has often been applied with the aim of distinguishing between chromosomally differentiated taxa. (Blackith and Blackith, 1969; Atchley, 1974; Atchley and Cheney, 1974). In these cases, the technique has been used in an attempt to distinguish between *a priori* karyotypic groups within the Morabine grasshoppers of Australia, which have a low level of morphological differentiation. It has also been applied to geographical species of the difficult genus *Chrotogonous* (Blackith and Kevan, 1967) and also has some application in the analysis of phase variation in locusts.

MATERIALS AND METHODS

Pinned and numbered specimens of all four races of *Caledia captiva*, as well as specimens of the morphologically distinct *Caledia species nova 1* from Papua (Table 6.1), were measured using a Zeiss dissecting microscope, equipped with an eyepiece graticule. All individuals had been cytologically examined or came from populations known to contain only one taxon.

Twelve characters were measured on each individual.

The characters chosen for analysis were:

1. Anterior femur length (A.F.) on external surface
2. Mid femur length (M.F.) on the anterior edge
3. Hind femur length (F)
4. Hind femur stripe width (S)
5. Hind femur width (W) - maximum
6. Elytron length (E) from the branching of the subcostal and costal veins to the apex.
7. Minimum pronotal width (Pm) between pronotal stripes at the middle.
8. Maximum pronotal width (Pr) between pronotal stripes at the rear.
9. Pronotal length (P) along median pronotal carina.

10. Distance between eyes at vertex (V).
11. Vertical diameter of the eye (O) - maximum.
12. Horizontal diameter of the eye (Oh) - minimum.

All measurements were made using a 10x eyepiece with a 0.8x objective. The data have been recorded and analysed as graticule units, 1 of which is equal to 0.8 mm. Because of the obvious sexual dimprohism in *Caledia*, males and females from each population have been measured and analysed separately.

Canonical variate analysis requires multivariate normally distributed data. The data from the first six populations measured, three each from the "Moreton" and "Torresian" populations, have been analysed using a basic statistics program, BASTATS, on the Univac 1108, to test for normality. There were no consistent significant deviations from normality in any of the six male and six female samples. Therefore the original and all subsequent data have been used in an untransformed state in the canonical analysis.

Canonical variate analysis was carried out using a modified Cooley and Lohnes program (Cooley and Lohnes, 1971), provided by Dr. M.T. Tanton (Forestry, S.G.S., A.N.U.) on the ANU Univac 1108 computer. Several different combinations of populations and races have been analysed. The results of a pilot analysis using three populations each of the "Moreton" and "Torresian" races are not presented here.

After each analysis, the population centroids for at least the first three variates have been plotted to determine the inter-taxon discrimination. Amongst other things, this allowed an assessment of the effect of the removal of characters from the analysis on the basis of their contribution to the first three discriminant functions.

Table 6.1.

Population	Race	Latitude°S	Collection Date
Species nova 1	species nova 1	8.69	26/9/1974
Daintree	Daintree	16.25	23/9/1975
Papua	Torresian	8.69	26/9/1974
Gove	Torresian	16.25	3/6/1975
Gin Gin	Torresian	24.98	27/2/1975
Bongmuller Crk	Torresian	26.07	13/6/1975
Gregors Crk	Torresian	26.95	9/6/1975
Brown Crk	Torresian	26.77	15/6/1975
Coles Crk	Moreton	26.33	28/2/1975
Scrubby Crk	Moreton	26.68	9/6/1975
Cooroy	Moreton	26.37	20/1/1975
Caloundra	Moreton	26.72	21/1/1975
Peachester	Moreton	26.80	14/3/1975
Sheepstation Crk	Moreton	26.88	15/6/1975
Telegraph Point	S.E. Aust.	31.28	13/10/1975
Wootton	S.E. Aust.	32.37	14/10/ 1975
Depot Beach	S.E. Aust.	35.63	2/1/1975

Populations used in multivariate analysis of morphological variation within and between races

RESULTS

1. Population Parameters - Character Means and Standard Deviations for all populations.

A total of 402 males and 383 females of all four races of *Caledia captiva* and also *Caledia species nova 1* have been measured. An examination of the population means and standard deviations (Table 6.2a,b) reveals that there is no clear cut pattern of inter-racial variation despite considerable variation between the population means. *C species nova 1*, which has a small body size and qualitative colour differences from *C. captiva*, generally has the smallest mean values for the characters, although the southernmost population of the "south east Australian" race, Depot Beach, has a smaller mean value for some characters, such as elytron length in the males. However given the overlap in the character means between the races in both the males and females, it is clear that a univariate analysis of the data will be of no use in distinguishing between the taxa and therefore has not been attempted. The statistics of the 16 populations of the four races of *C. captiva* give an indication of the overall similarity of the four races with some interpopulation, intra-racial variation. The only discernable trend is for an increase in population mean for most characters with a decrease in latitude.

Table 6.2a Means and Standard Deviations for Male Characters.

Populations	AF	MF	F	S	W	E	Pm	Pr	P	V	O	Oh
Species Nova 1	2.651	3.124	9.878	1.127	2.227	12.144	0.769	1.773	2.765	0.627	1.744	1.202
(45)	0.108	0.115	0.398	0.144	0.099	0.407	0.051	0.091	0.113	0.045	0.072	0.050
Daintree	2.450	3.261	11.661	1.550	2.483	14.400	0.861	1.939	3.111	0.733	1.828	1.267
(18)	0.129	0.142	0.524	0.253	0.099	0.872	0.061	0.085	0.076	0.049	0.107	0.069
Papua	2.800	3.512	12.030	1.367	2.505	14.940	0.892	2.057	3.395	0.867	1.880	1.285
(40)	0.138	0.162	0.577	0.200	0.108	0.636	0.057	0.106	0.185	0.047	0.099	0.062
Gove	2.577	3.343	11.290	1.690	2.510	14.303	0.883	2.063	3.250	0.800	1.863	1.233
(30)	0.141	0.183	0.570	0.202	0.127	0.565	0.065	0.103	0.133	0.037	0.089	0.071
Gin Gin	2.526	3.196	10.665	1.496	2.470	13.165	0.857	2.000	3.091	0.826	1.826	1.283
(23)	0.142	0.130	0.416	0.182	0.106	0.550	0.073	0.085	0.135	0.062	0.092	0.065
Bongmuller Ck	2.375	3.131	10.431	1.281	2.444	12.575	0.825	1.925	2.975	0.769	1.762	1.194
(16)	0.093	0.149	0.432	0.138	0.089	0.484	0.068	0.100	0.134	0.048	0.081	0.057
Gregors Ck	2.475	3.219	10.550	1.494	2.519	12.769	0.800	1.937	3.056	0.800	1.769	1.231
(16)	0.106	0.133	0.431	0.151	0.111	0.599	0.073	0.109	0.115	0.037	0.060	0.048
Brown Ck	2.383	3.177	10.283	1.133	2.400	12.439	0.811	1.833	2.939	0.783	1.728	1.222
(18)	0.092	0.142	0.391	0.168	0.124	0.584	0.058	0.103	0.154	0.038	0.083	0.055

Table 6.2a (contd)

Coles Ck	2.405	3.111	10.053	1.342	2.305	12.279	0.779	1.842	2.895	0.774	1.742	1.195
(19)	0.103	0.110	0.347	0.180	0.097	0.417	0.079	0.084	0.078	0.045	0.061	0.040
Scrubby Ck	2.370	3.057	10.183	1.509	2.300	12.191	0.783	1.800	2.852	0.765	1.687	1.217
(23)	0.088	0.090	0.219	0.138	0.067	0.424	0.065	0.100	0.112	0.049	0.069	0.039
Cooroy	2.337	2.993	9.897	1.243	2.260	12.130	0.823	1.797	2.823	0.780	1.740	1.207
(30)	0.119	0.134	0.485	0.168	0.104	0.625	0.068	0.116	0.125	0.041	0.067	0.045
Caloundra	2.519	3.207	10.800	1.522	2.411	13.170	0.833	1.878	3.048	0.859	1.793	1.270
(27)	0.136	0.124	0.599	0.183	0.122	0.533	0.083	0.089	0.165	0.050	0.073	0.054
Peachester	2.469	3.146	10.431	1.462	2.392	12.692	0.854	1.877	3.008	0.846	1.785	1.238
(13)	0.155	0.127	0.466	0.156	0.119	0.563	0.066	0.093	0.104	0.078	0.080	0.051
Sheepstation Ck	2.330	3.025	10.135	1.275	2.300	12.285	0.825	1.865	2.845	0.790	1.710	1.195
(20)	0.122	0.133	0.579	0.137	0.086	0.515	0.064	0.127	0.157	0.045	0.079	0.051
Telegraph Point	2.410	3.171	10.567	1.310	2.390	12.748	0.800	1.900	3.005	0.757	1.752	1.214
(21)	0.083	0.096	0.365	0.181	0.054	0.472	0.045	0.084	0.132	0.051	0.068	0.048
Wootton	2.321	3.017	10.046	1.179	2.275	12.462	0.837	1.787	2.933	0.771	1.687	1.192
(24)	0.114	0.143	0.425	0.159	0.107	0.674	0.071	0.085	0.163	0.062	0.068	0.050
Depot Beach	2.300	2.956	9.850	0.989	2.222	11.967	0.850	1.817	2.861	0.767	1.672	1.133
(18)	0.150	0.212	0.675	0.128	0.159	0.681	0.051	0.086	0.146	0.049	0.089	0.077

Table 6.2b Means and Standard Deviations for Female Characters.

Population	AF	MF	F	S	W	E	Pm	Pr	P	V	O	Ch
Species Nova 1	3.123	3.737	12.556	1.200	2.860	15.274	1.053	2.426	3.567	0.863	2.067	1.430
(43)	0.115	0.138	0.404	0.254	0.098	0.607	0.059	0.116	0.123	0.054	0.075	0.046
Daintree	2.896	3.874	14.174	1.700	2.935	17.826	1.078	2.526	3.861	0.948	2.109	1.452
(23)	0.136	0.236	0.819	0.276	0.180	1.035	0.074	0.121	0.190	0.051	0.100	0.059
Papua	3.130	4.030	14.520	1.547	3.057	18.152	1.165	2.627	4.200	1.142	2.157	1.490
(40)	0.152	0.168	0.701	0.282	0.152	0.644	0.083	0.148	0.209	0.068	0.075	0.059
Gove	3.096	3.974	14.165	2.062	3.078	17.578	1.157	2.578	3.996	1.104	2.152	1.470
(23)	0.197	0.228	0.925	0.272	0.278	1.152	0.084	0.254	0.343	0.082	0.159	0.106
Gin Gin	2.983	3.923	13.920	1.973	3.137	16.940	1.157	2.710	4.040	1.150	2.170	1.537
(30)	0.146	0.170	0.579	0.176	0.147	0.662	0.094	0.130	0.173	0.078	0.084	0.067
Bongmuller Ck	2.844	3.819	13.325	1.425	3.031	16.012	1.100	2.481	3.837	1.050	2.044	1.406
(16)	0.115	0.147	0.501	0.161	0.130	0.446	0.082	0.098	0.150	0.063	0.081	0.068
Gregors Ck	2.900	3.863	13.374	1.932	3.121	16.084	1.137	2.495	3.889	1.105	2.047	1.437
(19)	0.186	0.146	0.456	0.197	0.113	0.757	0.096	0.135	0.208	0.052	0.117	0.083
Brown Ck	2.813	3.800	12.973	1.493	3.000	15.707	1.060	2.427	3.687	1.047	2.040	1.427
(15)	0.099	0.136	0.482	0.162	0.100	0.595	0.112	0.088	0.196	0.064	0.091	0.046

Table 6.2b (contd)

Coles Ck	2.848	3.738	12.821	1.521	2.872	15.317	1.093	2.386	3.800	1.076	2.010	1.407
(29)	0.138	0.192	0.744	0.230	0.136	0.714	0.065	0.113	0.202	0.074	0.086	0.075
Scrubby Ck	2.790	3.717	12.997	1.890	2.870	15.027	1.103	2.373	3.660	1.030	2.010	1.410
(30)	0.132	0.168	0.528	0.183	0.102	0.686	0.081	0.131	0.133	0.060	0.071	0.055
Cooroy	2.763	3.668	12.716	1.558	2.837	15.395	1.089	2.453	3.716	1.047	2.058	1.426
(19)	0.142	0.214	0.746	0.313	0.142	0.828	0.129	0.131	0.195	0.051	0.102	0.081
Caloundra	3.050	4.006	14.469	1.931	3.031	16.906	1.062	2.526	4.100	1.212	2.181	1.544
(16)	0.126	0.161	0.584	0.154	0.120	0.300	0.102	0.077	0.141	0.072	0.091	0.063
Peachester	2.915	3.754	13.485	1.708	3.038	15.938	1.169	2.554	3.877	1.162	2.077	1.415
(13)	0.141	0.166	0.730	0.150	0.180	0.619	0.063	0.133	0.188	0.087	0.093	0.080
Sheepstation Ck	2.700	3.659	12.871	1.671	2.847	15.276	1.082	2.394	3.635	1.059	1.941	1.365
(17)	0.137	0.191	0.546	0.169	0.150	0.670	0.101	0.148	0.187	0.051	0.080	0.049
Telegraph Point	2.760	3.767	13.387	1.393	2.973	16.187	1.060	2.487	3.907	1.060	2.040	1.460
(15)	0.112	0.135	0.364	0.215	0.088	0.455	0.106	0.083	0.103	0.063	0.099	0.051
Wootton	2.740	3.693	12.940	1.493	2.900	15.760	1.147	2.393	3.867	1.047	2.007	1.407
(15)	0.140	0.116	0.608	0.234	0.151	0.732	0.130	0.133	0.176	0.052	0.096	0.059
Depot Beach	2.730	3.625	12.605	1.230	2.735	15.420	1.140	2.465	3.795	1.085	1.955	1.350
(20)	0.138	0.240	0.747	0.181	0.179	0.611	0.075	0.190	0.231	0.093	0.069	0.076

2. Canonical Variate Analysis

a) The "Moreton" and "Torresian" races (12 populations, 7 characters).

Five characters were excluded from the data after initial analysis since they were found to contribute very little to the interpopulation differentiation in the first three canonical variates. The same variables, namely mid femur length (2), minimum pronotum width (7), distance between eyes at the vertex (10) and both maximum and minimum diameter of the eye (11 and 12) were removed from both the male and female data. The first three canonical variates derived from the remaining 7 variables account for 93.21% of the interpopulation variation for the males and 89.46% for the females.

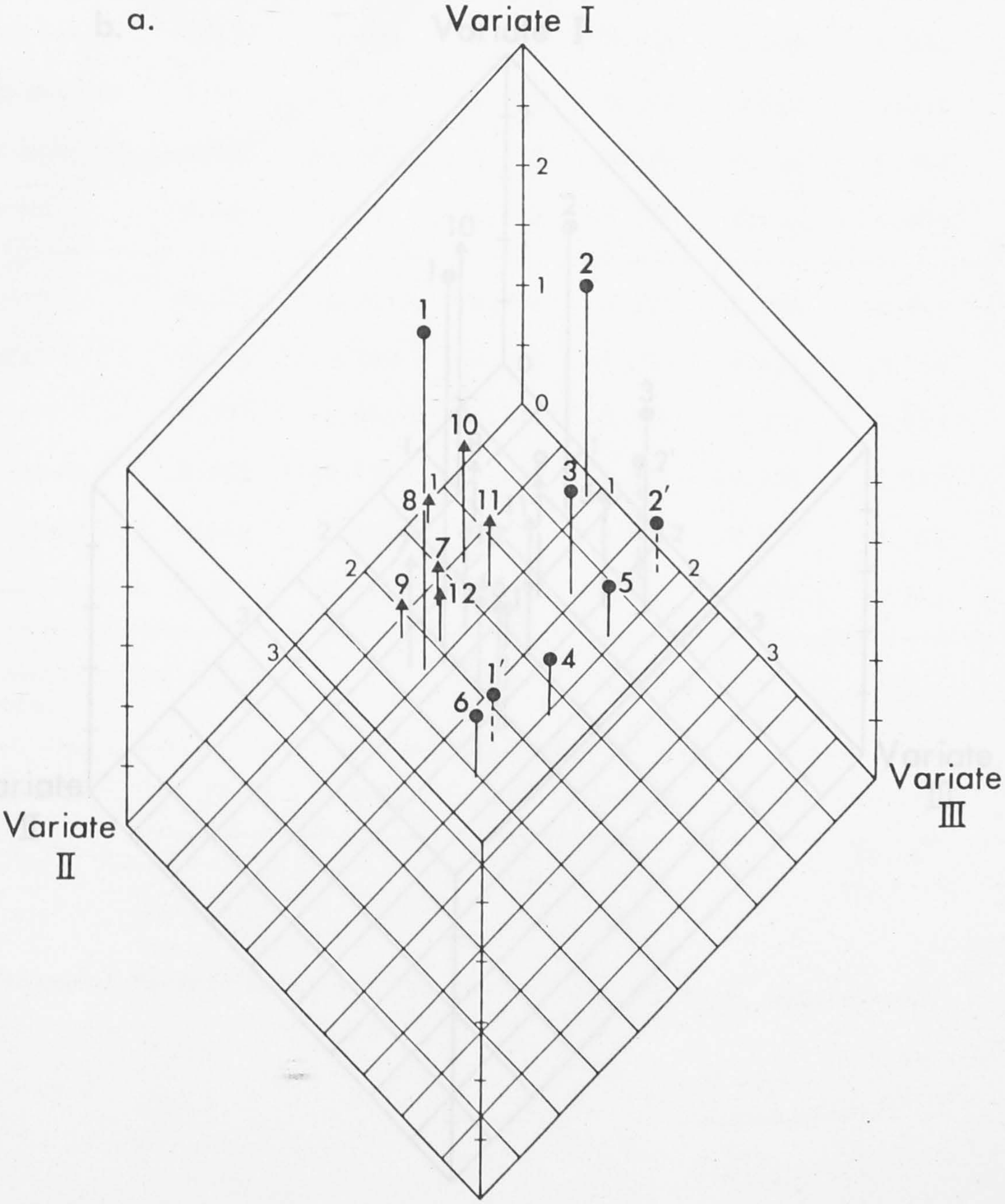
The exclusion of variables has its greatest effect in the analysis of the male data. In the initial analysis with all 12 variables included, there is no biologically meaningful ordering of populations according to taxa along any of the canonical axes. However the removal of the variables which contributed little to the first three variates has led to a non-overlapping ordering of the "Torresian" and "Moreton" populations along the third canonical axis. (Table 6.3, Fig 6.1a, b). A plot of the population centroids in three dimensional discriminant space (Fig. 6.1a), using arithmetically transformed axes so that all canonical variate means are positive, reveals the nature of the interpopulation discrimination for the males. The Gove and Papuan populations of the "Torresian" race are discriminated from the "Moreton" populations, and indeed the "Torresian" populations from south east Queensland, along the first canonical axis. The populations of the "Moreton" and "Torresian" races from south east Queensland on the other hand are discriminated from each other along the third canonical axis.

In the analysis of the female data, with all 12 character variables

Figure 6.1. Plot of first three canonical variate means for 12 group analysis.

(a) Male means, 7 variables; (b) Female means, 7 variables.

Populations are numbered according to Table 6. Circles symbolise "Torresian" populations and triangles "Moreton" populations. The 1' and 2' populations are the transformed population means for the Papuan and Gove populations, after removal of the effect of latitude on the first and third canonical variates.


a.
Variate I
2
1
0
1
2
10
8
1
11
3
1
2′
7
2
9
12
5
2
3
4
3
1′
6
Variate II
Variate III

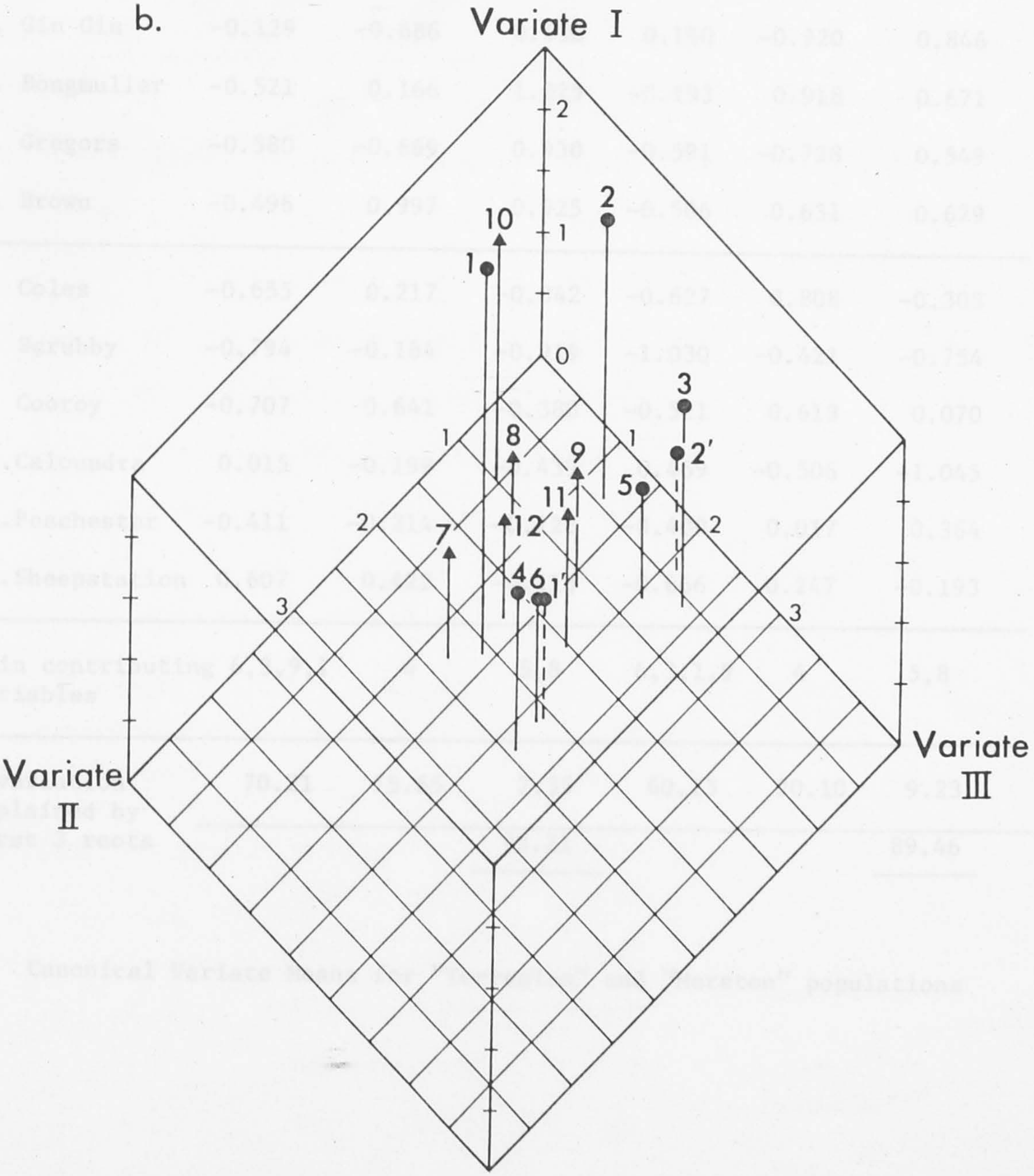

b.
Variate I
Variate II
Variate III
2
1
0
1
2
3
1
2
3
1
2
10
1
8
9
11
5
3
2'
12
7
4
6
1'

Table 6.3

	Male			Female		
Population	I	II	III	I	II	III
1. Papua	1.869	0.689	-0.043	1.658	0.578	-0.108
2. Gove	0.791	-1.369	-0.045	0.791	-1.071	-0.273
3. Gin Gin	-0.129	-0.686	0.438	0.180	-0.920	0.846
4. Bongmuller	-0.521	0.166	1.025	-0.193	0.918	0.671
5. Gregors	-0.580	-0.669	0.930	-0.591	-0.728	0.549
6. Brown	-0.496	0.997	0.925	-0.506	0.631	0.629
7. Coles	-0.655	0.217	-0.342	-0.627	0.808	-0.305
8. Scrubby	-0.794	-0.184	-0.919	-1.030	-0.421	-0.754
9. Cooroy	-0.707	0.641	-0.380	-0.521	0.613	0.070
10.Caloundra	0.015	-0.199	-0.439	0.469	-0.506	-1.045
11.Peachester	-0.411	-0.214	-0.128	-0.400	0.017	0.364
12.Sheepstation	0.607	0.422	-0.118	-0.666	0.247	-0.193
Main contributing variables	6,3,9,1	4	5,8	6,3,1,9	4	5,8
% variation explained by first 3 roots	70.21	15.65	7.35	60.13	20.10	9.23
			93.21			89.46

Canonical Variate Means for "Torresian" and "Moreton" populations

Table 6.4

Factor pattern for discriminant functions

a) Male

	Variate		
Character	I	II	III
1	.836	-.074	.047
3	.891	-.129	.115
4	.198	-.871	-.306
5	.518	-.404	.631
6	.956	-.228	.093
8	.662	-.399	.380
9	.861	-.238	.251

b) Female

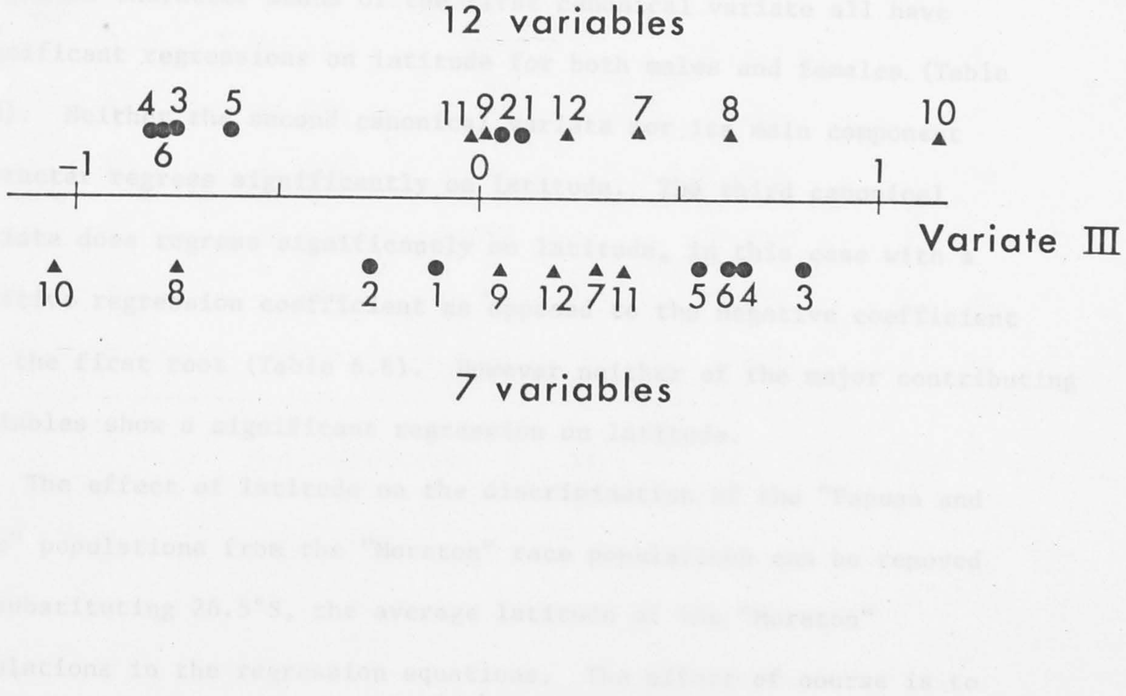
	Variate		
Character	I	II	III
1	.734	-.263	-.033
3	.757	-.326	-.052
4	-.035	-.976	-.144
5	.388	-.400	.548
6	.943	-.236	.161
8	.543	-.337	.498
9	.733	-.173	.103

included, there is a good inter-racial differentiation of the south east Queensland populations of the "Moreton" and "Torresian" races. However the Papuan and Gove third canonical variate means overlap the means for the Cooroy and Peachester populations of the Moreton race. The removal of the same five variables which were excluded from the male data does not improve the ordering of the Papuan and Gove populations along the third axis. Indeed, if anything the removal of the variables worsens the inter-racial discrimination, particularly for the south east Queensland populations (Fig. 6.2), although the population centroids still do not overlap in the third axis. Therefore the discriminant function vector for all 12 variables (0.016, -0.094, 0.038, 0.098, -0.559, -0.196, -0.212, -0.449, -0.070, 0.138, -0.113, -0.089) separates the races better than the third discriminant function with only 7 variables (Table 6.4b).

For the purpose of comparison with the results of the analysis of the male data, the population centroids derived from the 7 variable analysis have been plotted in 3D discriminant space (Fig. 6.1b). The results are similar to the male results except of course that there is more overlap in the third axis. Again the Gove and Papuan populations have the largest first canonical variate means although the Caloundra population of the "Moreton" race also has a large first canonical variate value.

The pattern of contribution of the characters to the canonical variates can be gauged from the factor pattern of the discriminant function (Table 6.4a, b). In the third axis, which is the main axis of racial discrimination, two width characters, width of femur (5) and maximum width of pronotum (8), are the main contributors in both the males and females. In fact these characters have the largest factors regardless of whether 7 or 12 variables are included in the female analysis.

Figure 6.2. A comparison of the third canonical variate means for females with 12 variables included and with only 7 variables included in the analysis.



12 variables
4
3
5
6
11
9
2
1
12
7
8
10
−1
0
1
Variate III
10
8
2
1
9
12
7
11
5
6
4
3
7 variables

Variation within the "Torresian " Race - An effect of latitude

A superficial examination of the "Torresian" grasshoppers reveals that the more northern population samples contain individuals with considerably larger overall body size than the southern population samples from south east Queensland. Since the six "Torresian" populations used here were collected over a wide latitudinal range from 8.69°S to 26.95°S, regression analysis provides a method of examining the effect of latitude on both the canonical variate means and the character means. In particular, the first canonical variate is often considered to reflect size differences in canonical analysis (Campbell, pers.com) and indeed appears to be related to size in this case.

The first canonical variate means as well as the four major component character means of the first canonical variate all have significant regressions on latitude for both males and females (Table 6.5). Neither the second canonical variate nor its main component character regress significantly on latitude. The third canonical variate does regress significantly on latitude, in this case with a positive regression coefficient as opposed to the negative coefficient for the first root (Table 6.6). However neither of the major contributing variables show a significant regression on latitude.

The effect of latitude on the discrimination of the "Papuan and Gove" populations from the "Moreton" race populations can be removed by substituting 26.5°S, the average latitude of the "Moreton" populations in the regression equations. The effect of course is to lower the Papuan and Gove centroids on the first canonical axis, but to move them closer to the south east Queensland populations of the "Torresian" race along the third axis, which is the main axis of inter-racial differentiation. The expected first canonical variate means are -0.495 for the males and -0.316 for the females compared with 1.869 and

Table 6.5 Regression analysis of "Torresian" populations - Canonical Variates and Morphological Characters on Latitude

	Males		Females	
Character	F ratio	Signif	F ratio	Signif.
Canon Var 1	482.267	***	75.207	***
2	0.008	NS	0.006	NS
3	15.177	*	11.073	*
Character 6	82.538	***	26.220	**
3	160.531	***	14.922	*
9	47.096	**	7.264	*
1	26.246	**	16.918	**
4	0.406	NS	0.0003	NS
5	1.396	NS	0.008	NS
8	6.446	NS	1.115	NS

N.S. not significant * 5%, ** 1%, *** 0.1%

Table 6.6 Regression equations for canonical variates on latitude for six "Torresian" populations

Variate	Sex	Equation
I	Male	$y = 2.977 - 0.131\,x$
I	Female	$y = 2.652 - 0.112\,x$
III	Male	$y = -0.731 + 0.059\,x$
III	Female	$y = -0.748 + 0.052\,x$

Table 6.7 Regression analysis - Canonical Variate 1 on Frequency of Acrocentric X in "Moreton" populations.

Population	Frequency of Acrocentric X	Canon Var I Female	Canon Var I Male
Scrubby	0	-1.030	-0.794
Sheepstation	0	-0.666	-0.607
Coles Creek	0.395	-0.627	-0.655
Cooroy	0.796	-0.521	-0.707
Peachester	0.667	-0.400	-0.411
Caloundra	1.000	0.469	0.015
F ratio (1, 5)		8.190	3.619
Signif.		*	NS

Regression equation for females

$$y = -0.932 + 0.987\,x$$

1.685 for the Papuan population and 0.791 and 0.791 for the Gove population males and females respectively. For the third canonical variate, the expected values are 0.832 for the males compared with observed values of -0.043 and -0.045 for Papua and Gove respectively and 0.630 for the females compared with -0.108 and -0.273. The overlap of third canonical variate means between races is completely removed for the females and for both males and females the inter-racial discrimination along the third canonical axis is distinct and complete (Fig. 6.1a, b).

Variation within the "Moreton" race - an X chromosome effect

Because the six "Moreton" populations lie between 26.33°S and 26.88°S, it is not feasible to test the regression of canonical variate means on latitude. However the wide disparity in the first canonical variate means between Caloundra (10) and Scrubby Creek (8), which are situated at 26.72°S and 26.68°S respectively, demonstrates clearly that no relationship exists between first canonical variate and latitude similar to the relationship found in the "Torresian" race. Two important differences exist between the Caloundra and Scrubby Creek populations. First there is a fixed difference in X chromosome morphology. The Scrubby Creek population has a metacentric X whereas the Caloundra population is fixed for an acrocentric X chromosome (see Chapter II). The second difference is the more western, inland position of the Scrubby Creek population compared with the coastal position of the Caloundra collecting site.

A regression analysis of the first canonical variate means on the frequency of the acrocentric form of the X chromosome (Table 6.7) demonstrated a significant relationship in the females but not in the males. However the confounding effect of autosomal polymorphism has not been taken into account. The differences in size which are reflected in the differences in the first canonical variate means are qualitatively detectable in populations which are fixed on the alternative forms of the X chromosome.

b) All four races of *Caledia captiva* (16 populations)

The initial analysis with all 12 variables included led to a clear differentiation of the "Daintree" race from the other three races of *Caledia captiva*. For both the males and females there is clear discrimination along the third axis. Further, in the males, the "Daintree" population is also differentiated in the fourth axis (Fig. 6.3a, b). The discriminant function vector for the third canonical axis in the females is (0.456, 0.275, 0.246, -0.037, 0.274, 0.208, 0.289, 0.393, 0.555, 0.875, 0.309, 0.310). For the males, the third and fourth discriminant function vectors are (0.466, 0.215, 0.030, 0.035, -0.001, 0.032, 0.054, 0.080, 0.212, 0.684, 0.148, 0.295) and (-0.133, -0.170, 0.044, 0.204, -0.258, 0.002, -0.070, -0.395, -0.237, 0.065, -0.123, 0.280) respectively.

The "Torresian", "Moreton" and south east Australian races are not differentiated from each other in the 16 group analysis with all 12 variables included. In an attempt to improve the discrimination between these three races, character variables which contribute little to the interpopulation discriminations in the first three or four roots have been removed. The same six characters have been excluded from the male and female data. The eliminated variables are mid femur length (2), femur width (5), minimum pronotum width (7), maximum pronotum width (8) and the maximum and minimum diameter of the eye (11 and 12). The first three canonical roots derived from these six variables account for 94.07% of the interpopulation variation in the case of males and 92.40% in the case of the females. The removal of these six variables has led to only a marginal improvement in the

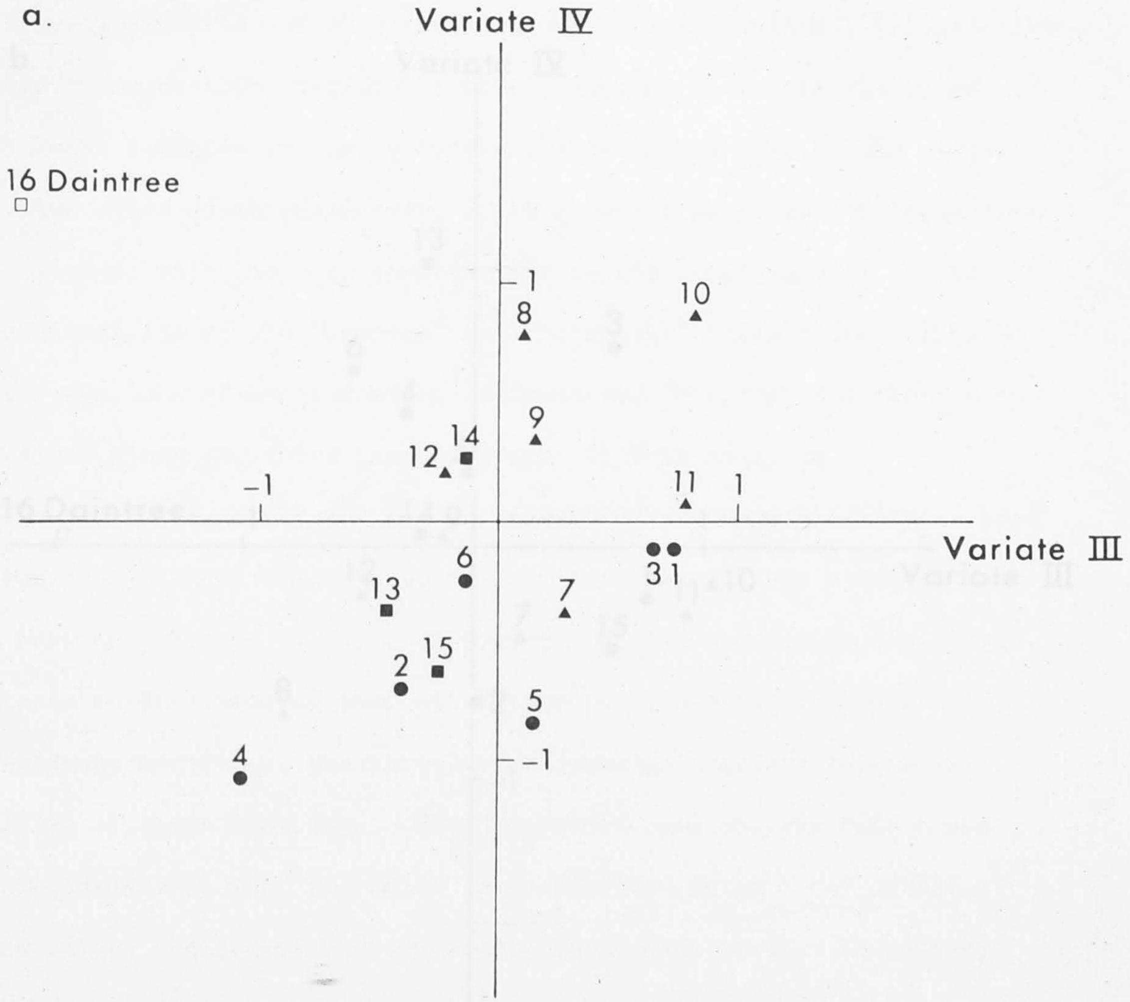
Figure 6.3. Third and fourth canonical variate means for the 16 group analysis.

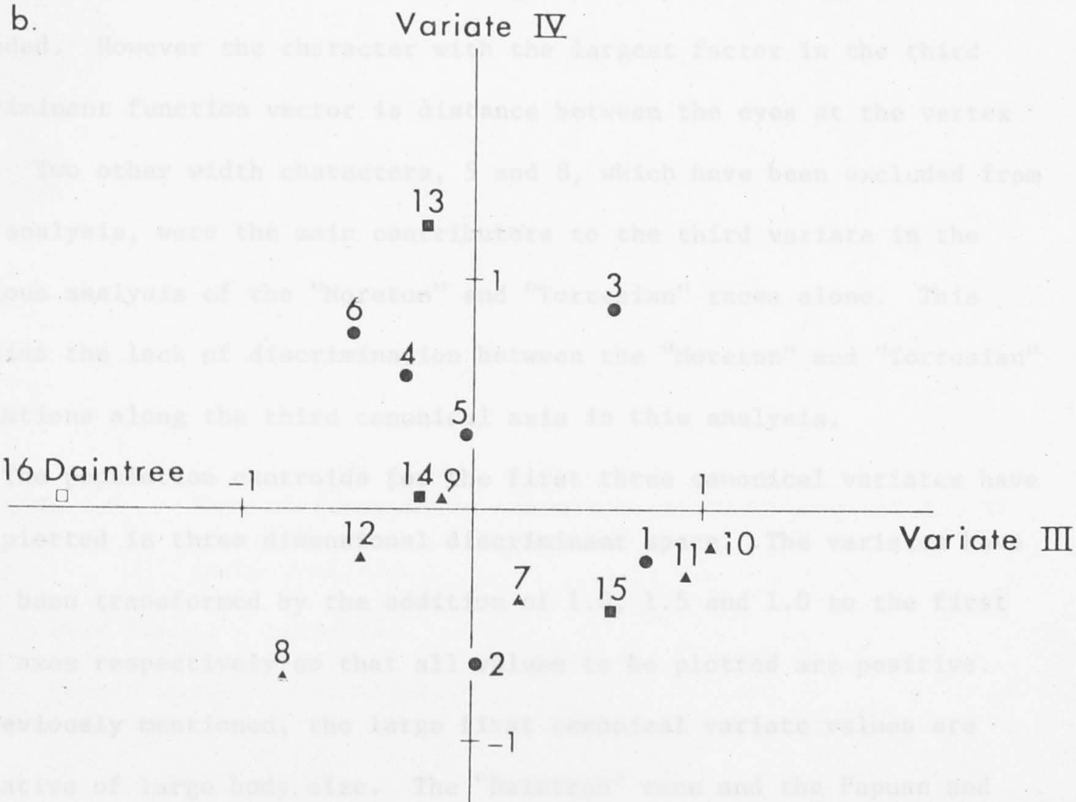
(a) Male means, 12 variables.

(b) Female means, 12 variables.

Note that in the case of the males, discrimination of the "Daintree" population occurs in both the third and fourth variates, whereas in the females it is differentiated only in the third canonical axis.

Closed squares - "South east Australian" populations.


a.
Variate IV
16 Daintree
1
10
8
9
14
12
−1
11
1
Variate III
6
3 1
13
7
15
2
5
−1
4


b.
Variate IV
13
1
3
6
4
5
16 Daintree
–1
14
9
1
12
1
11
10
Variate III
7
15
8
2
–1

discrimination between the three similar races. However it has led to no deterioration in the differentiation of the "Daintree" race from them. The exclusion of variables therefore has at least simplified the discrimination of the "Daintree" race from the other three races.

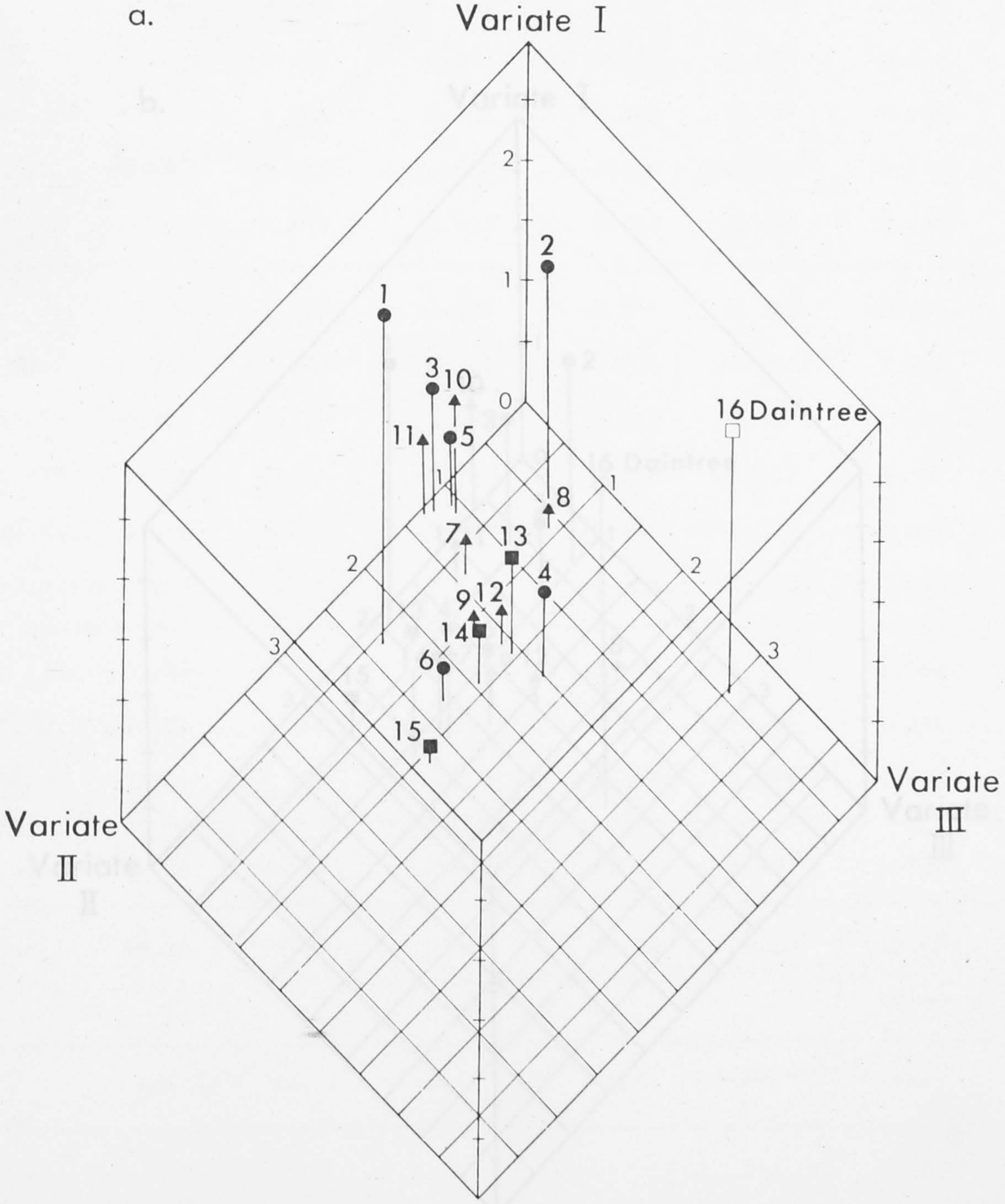
The main axis of inter-racial discrimination in the six variable analysis is the third canonical axis (Table 6.8) in the case of the males and the females. The pattern of contribution of variables to the first three canonical variates (Table 6.9 a, b) is similar to the pattern in the 12 group analysis and also the 16 group analysis with all 12 variables included. However the character with the largest factor in the third discriminant function vector is distance between the eyes at the vertex (10). Two other width characters, 5 and 8, which have been excluded from this analysis, were the main contributors to the third variate in the previous analysis of the "Moreton" and "Torresian" races alone. This explains the lack of discrimination between the "Moreton" and "Torresian" populations along the third canonical axis in this analysis.

The population centroids for the first three canonical variates have been plotted in three dimensional discriminant space. The variates have first been transformed by the addition of 1.0, 1.5 and 1.0 to the first three axes respectively so that all values to be plotted are positive. As previously mentioned, the large first canonical variate values are indicative of large body size. The "Daintree" race and the Papuan and Gove populations of the "Torresian" race all have large first canonical variate values and indeed have obviously large body sizes. Large body size and large first canonical variate in the "Torresian" populations are related to the more northern position of populations such as Papua and Gove. It is likely therefore that the large size of the "Daintree" race grasshoppers from northern Queensland is a reflection of their latitudinal position, although there may be genetic components of the size difference also.

Figure 6.4. Plot of first three canonical variate means for the 16 group analysis.

(a) Male means, 6 variables.

(b) Female means, 6 variables.


a.
Variate I
Variate II
Variate III
0
1
2
3
1
2
3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16 Daintree

b.

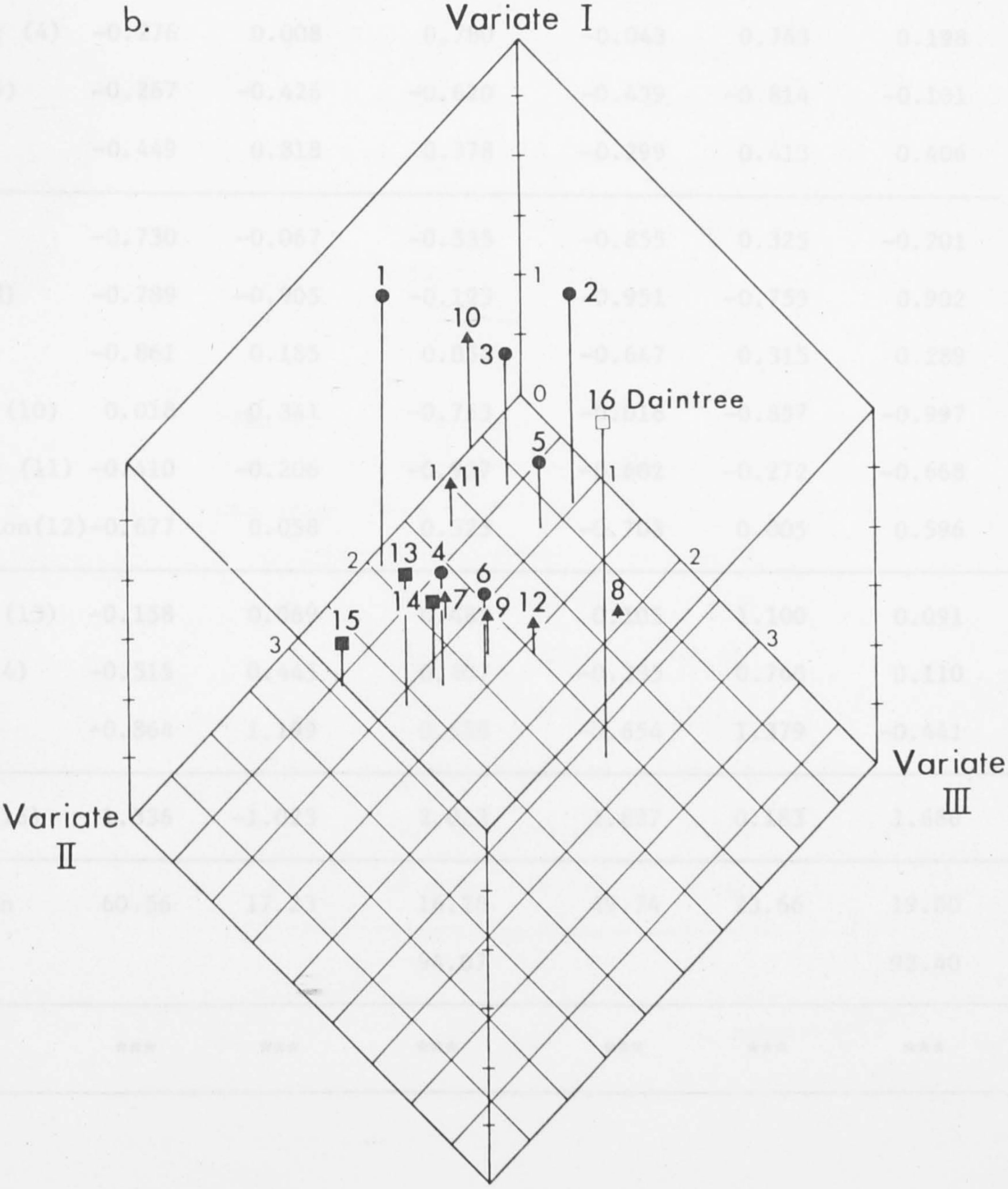

Variate I
Variate II
Variate III
0
1
2
3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16 Daintree

Table 6.8

	Male Variate Means			Female Variate Means		
Population	I	II	III	I	II	III
Papua (1)	1.745	1.042	-0.387	1.307	0.392	-0.898
Gove (2)	1.004	-1.052	-0.262	0.785	-1.189	-0.008
Gin Gin (3)	0.012	-0.265	-0.825	0.112	-0.880	-0.544
Bongmuller (4)	-0.276	0.008	0.780	-0.043	0.763	0.198
Gregors (5)	-0.267	-0.426	-0.620	-0.439	-0.814	-0.101
Brown (6)	-0.449	0.818	0.378	-0.299	0.413	0.406
Coles (7)	-0.730	-0.067	-0.335	-0.855	0.325	-0.201
Scrubby (8)	-0.789	-0.905	-0.123	-0.951	-0.759	0.902
Cooroy (9)	-0.861	0.185	0.054	-0.647	0.315	0.289
Caloundra (10)	0.018	-0.341	-0.753	-0.016	-0.857	-0.997
Peachester (11)	-0.410	-0.206	-0.927	-0.602	-0.272	-0.668
Sheepstation(12)	-0.677	0.058	0.325	-0.703	0.005	0.596
Telegraph (13)	-0.158	0.069	0.483	0.105	1.100	0.091
Wootton (14)	-0.515	0.445	0.407	-0.335	0.760	0.110
Depot (15)	-0.864	1.199	0.559	-0.654	1.379	-0.441
Daintree (16)	1.336	-1.023	2.023	1.827	0.183	1.680
% Variation	60.56	17.23	16.28	49.74	23.66	19.00
			94.07			92.40
Signif.	***	***	***	***	***	***

Table 6.9 Factor Pattern for Discriminant Functions for all four races of *Caledia captiva*.

a)

Male	Variate		
Character	I	II	III
1	0.782	0.146	-0.484
3	0.928	-0.036	-0.065
4	0.395	-0.837	-0.347
6	0.982	-0.057	-0.090
9	0.864	0.069	-0.286
10	0.299	0.199	-0.635

b)

Female	Variate		
Character	I	II	III
1	0.563	-0.404	-0.524
3	0.713	-0.345	-0.320
4	0.074	-0.962	0.058
6	0.929	-0.189	-0.306
9	0.532	-0.126	-0.636
10	-0.003	-0.286	-0.889

The three populations of the "south east Australian" race cluster reasonably closely in the three dimensional discriminant space. In the case of the males, there is a non overlapping ordering of the "south east Australian" and "Moreton" populations along the third axis and along the second canonical axis in the case of the females (Fig. 6.4a, b). However they are not clearly differentiated from the "Torresian" populations. A satisfactory discrimination between the "Torresian" and "south east Australian" races could probably be expected in a separate analysis of these two races alone, since the inclusion of extreme groups such as the "Daintree" population decreases the ability of canonical analysis to resolve less extreme groups (Thorpe, 1976).

The size of the first canonical variates in the "south east Australian" populations is also clearly related to latitude. The Telegraph Point (13) population has the largest first variate value and the most northern position at 31.28°S whereas the Depot Beach (15) population at 35.63°S has the smallest first variate. Body size is therefore correlated with latitude in the "south east Australian" race. A similar relationship was demonstrated in the "Torresian" populations.

c) Four races of *Caledia captiva* and *C.* species *nova 1* (17 groups)

Caledia species *nova 1* is qualtitatively different from the races of *C. captiva* in colour and morphology. The hind femora in *C.* species *nova 1* are orange compared with yellow in *C. captiva* and the body size is considerably smaller on average. The extent of the morphological differentiation between *C.* species *nova 1* and the races of *Caledia captiva* is reflected in the ease with which they are differentiated in the canonical variate analysis. Even in the analysis with all 12 variables included *C.* species *nova 1* is clearly discriminated in the first canonical axis, which is the axis of maximum interpopulation variation.

TABLE 6.10

Canonical Variate Means for the 17 Group Analysis

	Male Variate Means			Female Variate Means		
Population	I	II	III	I	II	III
Papua (1)	-1.371	1.488	1.089	-0.741	1.351	0.433
Gove (2)	-1.066	0.330	-1.064	-0.446	0.657	-1.285
Gin Gin (3)	-0.257	-0.425	-0.173	-0.675	-0.276	0.738
Bongmuller (4)	0.080	-0.613	-0.526	0.045	-0.222	0.501
Gregors (5)	0.032	-0.787	-0.598	-0.204	-0.902	-0.958
Brown (6)	0.238	-0.537	0.336	0.219	-0.492	0.112
Coles (7)	0.405	-0.519	0.090	0.171	-0.765	0.406
Scrubby (8)	0.301	-0.727	-0.435	0.133	-0.930	-0.749
Cooroy (9)	0.392	-0.670	0.393	0.013	-0.574	0.427
Caloundra (10)	-0.473	-0.489	0.514	-0.987	-0.107	-0.478
Peachester (11)	-0.112	-0.805	0.503	-0.335	-0.829	-0.096
Sheepstation(12)	0.148	-0.722	0.107	-0.236	-0.750	0.100
Telegraph (13)	-0.016	-0.205	-0.387	-0.452	-0.064	1.033
Wootton (14)	0.080	-0.462	0.484	-0.274	-0.428	0.748
Depot (15)	0.450	-0.453	0.918	-0.236	-0.379	1.777
Daintree (16)	-1.264	0.752	-1.749	-0.478	1.942	-0.224
Species nova(17)	1.880	1.493	-0.230	2.362	0.553	-0.103
% variation	43.49	29.91	10.39	45.37	27.57	11.45
			83.79			84.39
Signif.	***	***	***	***	***	***

TABLE 6.11

Factor Pattern for Discriminant Functions

(a)

Male Character	Variate I	 II	 III
1	-.196	.775	.144
3	-.800	.437	-.054
4	-.541	-.084	-.499
5	-.656	-.089	-.233
6	-.819	.509	-.076
8	-.662	.228	-.117
9	-.804	.316	.080
10	-.728	-.311	.473

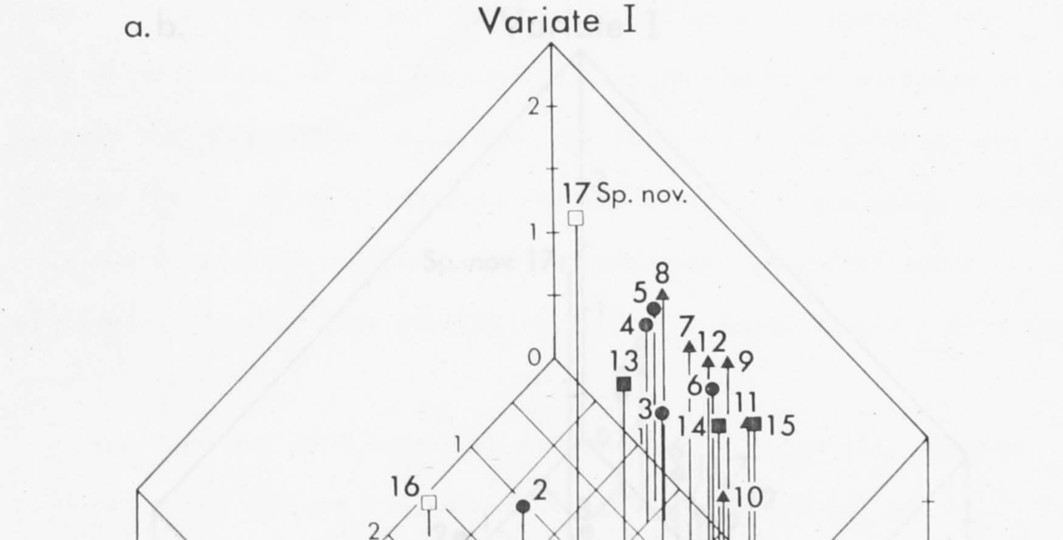
(b)

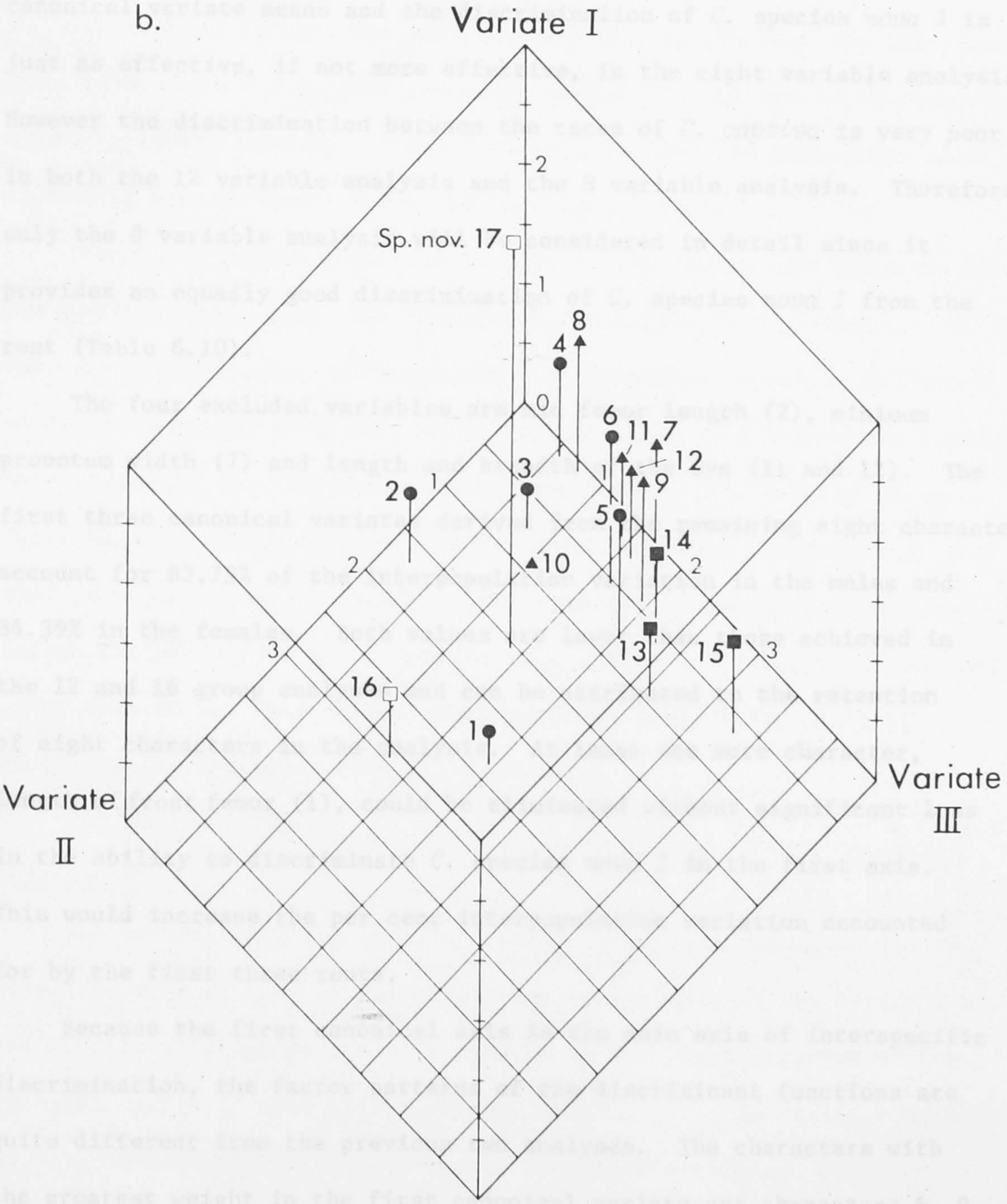
Female Character	Variate I	 II	 III
1	.188	.545	-.389
3	-.566	.504	-.330
4	-.516	-.142	-.805
5	-.322	.138	-.448
6	-.530	.734	-.226
8	-.332	.312	-.197
9	-.596	.322	-.055
10	-.767	-.218	-.051

Figure 6.5. Plot of first three canonical variate means for 17 group analysis.

(a) Male means, 8 variables.

(b) Female means, 8 variables.


a.
Variate I
2
1
0
17 Sp. nov.
8
5
4
7
12
9
13
6
3
11
14
15
10
16
2
1
1
2
3
4
3
4
Variate II
Variate III


b.
Variate I
Variate II
Variate III
Sp. nov. 17
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16

The differences between *C.* species *nova 1* and *C. captiva* are therefore large relative to the extensive non-racial variation within *C. captiva*.

Four characters have been removed from the analysis after the discriminant function vectors derived from the initial analysis indicated that they had little weight in the first three canonical variates. Of course, this exclusion has little effect on the first canonical variate means and the discrimination of *C.* species *nova 1* is just as effective, if not more effective, in the eight variable analysis. However the discrimination between the races of *C. captiva* is very poor in both the 12 variable analysis and the 8 variable analysis. Therefore only the 8 variable analysis will be considered in detail since it provides an equally good discrimination of *C.* species *nova 1* from the rest (Table 6.10).

The four excluded variables are mid femur length (2), minimum pronotum width (7) and length and breadth of the eye (11 and 12). The first three canonical variates derived from the remaining eight characters account for 83.79% of the interpopulation variation in the males and 84.39% in the females. Both values are lower than those achieved in the 12 and 16 group analyses and can be attributed to the retention of eight characters in the analysis. At least one more character, length of front femur (1), could be eliminated without significant loss in the ability to discriminate *C.* species *nova 1* in the first axis. This would increase the per cent interpopulation variation accounted for by the first three roots.

Because the first canonical axis is the main axis of interspecific discrimination, the factor patterns of the discriminant functions are quite different from the previous two analyses. The characters with the greatest weight in the first canonical variate are characters 6, 9, 3 and 10 (Table 6.11a, b). Character 10 is a width measurement whereas the other characters are length measures. Three length characters, 1,6 and 3, have the largest weighting in the second canonical variate, and characters 4 and 10 have the largest factors in the third discriminant function.

The population centroids for the first three canonical variates have been plotted in three dimensional discriminant space and illustrate the significant discrimination of *C.* species *nova 1* from the rest (Fig. 6.5a, b). In contrast with the previous two analyses however, the populations with the largest first canonical variates, such as *C.* species *nova 1*, have the smallest body sizes. Further the relationship between size and first canonical variate is not nearly so clear in these analyses, although populations such as Gove and Papua have small first canonical variate means and large body measurements.

The inter-racial discrimination in *C. captiva* is poor relative to the previous analyses. The "Daintree" population is discriminated in the second and third axes, although the discrimination is not as effective as in the 16 group analysis. With the exception of the Gove and Papuan populations of the "Torresian" race, populations of the other three races cluster fairly closely in the second and third dimensions of the plot for both males and females. Consequently it is not possible to assess the affinities of *C.* species *nova 1* with the races of *C. captiva*, other than to say that on the given evidence (Fig. 6.5a, b) it is about equally different from all four races.

b) *The Phallic Complex*

Introduction

The structure of the phallic complex is a vital character for the classification of many of the higher taxonomic units of the Acridoidea, and additionally can be usefully applied to the classification of lower taxonimic units in some groups (Dirsh, 1956). It is generally considered to be a conservative structure in the subfamily Acridinae, which contains the genus *Caledia*. However there are sufficient differences in the size and shape of the epiphallus to characterize at least some genera within it (Dirsh, 1956).

The phallic complex has been used for categorising males of species which are otherwise morphologically indistinguishable. For example, three sibling species of the *Alutacea* group of the genus *Schistocerca* in north America can be recognised on qualitative differences in the structure of the phallic complex (Hubbel, 1960). However *Schistocerca* belongs to the subfamily Cyrtacanthacridinae in which the phallic complex is more variable than in the Acridinae.

The aim of the present study was to determine whether the "Moreton" and "Torresian" races of *Caledia captiva* could be distinguished on qualitative differences of the phallic complex. Such qualitative differences, if they existed, among other things, would facilitate further detailed mapping of the hybrid zone, because they could be used as criteria for selecting population sampling sites for subsequent chromosomal analyses. For comparative purposes, several males of the "Daintree" race were also examined.

Materials and Methods:

The pinned bodies of males (Table 6.12), which had previously been karyotyped, were relaxed overnight in a moistening chamber. Using very fine dissecting needles and watch makers forceps, the phallic complexes were carefully removed so that the external appearance of the grasshopper was not distorted. The complexes were placed in cold 10% KOH for several hours or overnight to break down the soft tissue. This mild form of treatment minimises the distortion of the sclerotised parts of the complex.

Table 6.12

Race	Population	Individuals
"Torresian"	T1P3	6
"Torresian"	Appletree Creek	2
"Moreton"	T1P4	5
"Moreton"	Spring Valley Creek	3
"Moreton"	Tuan	5
"Daintree"	Daintree	4

Populations from which a sample of phallic complexes were examined.

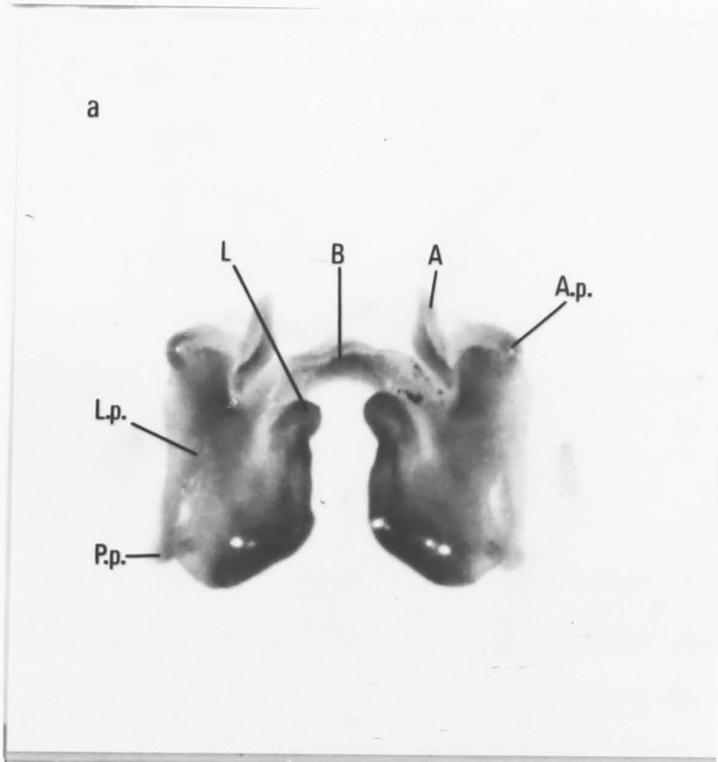
Figure 6.6. Epiphallus of "Torresian" male of *Caledia captiva*

(a) Dorsal view with anterior end oriented towards the top of the figure.

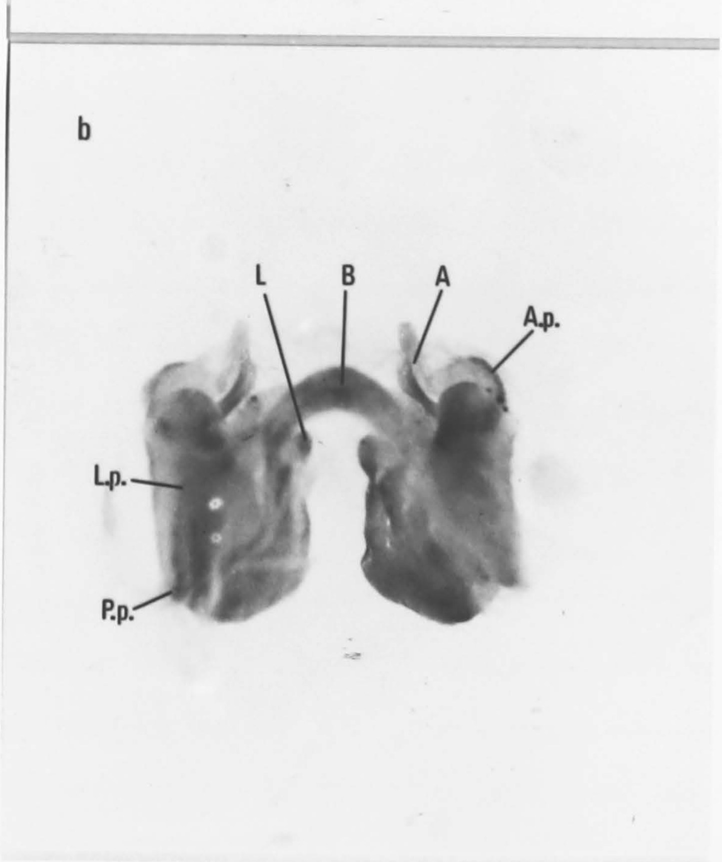
(b) Ventral view of epiphallus. Membranes attach this side of the epiphallus to the rest of the phallic complex.

P.p. - Posterior projection; L.p. - Lateral plate; L - Lophus;
B - Bridge; A - Ancora; A.p. - Anterior projection.

a



b



Extraneous tissue was carefully removed from the complexes using a fine brush and they were finally stored in 70% ethanol. (Procedure from Dirsh, 1956 and Key, personal communication).

All dissected complexes were examined under a dissecting microscope. A representative sample of epiphalli and complexes without epiphallus were photographed using incident and transmitted light at 6.4 X magnification on a Zeiss Tessovar using Plus X Pan Film. Enlargements were made at identical magnification to allow detailed side by side comparison of the prints.

Results:

The sclerortised parts of phallic complex, without epiphallus, are indistinguishable in the "Moreton", "Torresion" and "Daintree" races. There is some size variation but this equally distributed both within and between the races and is to be expected, given the overall variation in body size.

The epiphallus is generally more useful in Acridine taxonomy. However in this case there are no obvious differences in the structure of the epiphallus which allow recognition of the races. The ancorae and lophi are of similar shape and size in all three races (Fig. 6.6). There is some variation in the size of the lateral plates of the epiphalli, but again the level of intra-racial variation precludes any inter-racial distinctions.

Discussion:

The technique of canonical variate analysis has detected varying degrees of inter-racial differentiation and revealed some of the morphological affinities among the chromosomal races of *Caledia captiva*. In particular, the "Moreton" and "Torresian" races can be differentiated in discriminant space, mainly in the third canonical variate which is the main axis of inter-racial discrimination. The Papuan and Gove populations of the "Torresian" race are distinguished from the "Moreton" populations mainly in the first canonical variate, which is related to size. However both the first and third variates in the "Torresion" race are correlated with latitudinal position. If the effect of latitude is removed from the Papuan and Gove populations, they differ from the "Moreton" populations in the third canonical variate rather than the first variate. The differentiation detected is not particularly useful taxonomically except for populations of the two races collected in the same geographical region, such as the south east Queensland region. The removal of the effect of latitude from the Papuan and Gove populations by using regression equations requires that they already be recognised as "Torresian" populations. This precludes the use of the discriminant functions in identifying "Torresian" populations from other regions unless both the first and third canonical variate means are carefully considered.

The character variables with the heaviest weighting in the third discriminant function are characters 5 and 8, which are width of femur and maximum width of pronotum respectively. Even for these characters, there is overlap in the population means between the races for both the males and females. Clearly the distinction is not based simply on the size of these characters but on their inter-relationships and covariation with other characters. The discrimination in the female 8 variable analysis is less satisfactory than in the equivalent male analysis. However this can be attributed to the removal of characters since the discrimination between the south east Queensland populations of the two races was satisfactory when all variable were retained.

The affinity of the "Moreton" populations with the "Torresian" race fits reasonably well with their geographical proximity to the contact zone and the known frequency of acrocentric and telocentric chromosomes within them which are assumed to be derived from introgression. For example, the Caloundra and Scrubby Creek populations of the "Moreton" race differ most from the "Torresian" race along the third canonical axis (Table 6.3) and likewise have the lowest frequency of acrocentric and telocentric chromosomes. However the range of chromosome frequencies and the number of populations studied does not allow a thorough morphological test of the introgression hypothesis.

The percentage interpopulation variation accounted for by the third canonical variate in the comparison of the "Torresian" and "Moreton" populations alone is 7.35% for the males and 9.23% for the females. Since this is the main axis of inter-racial differentiation, these values give a crude estimate of the amount of inter-racial differentiation relative to other interpopulation variation. For example, 70% of the interpopulation variation is accounted for by the first variate in the males, and 60% in the females. However the major components of the first variate are geographical variation within the "Torresian" race and genetic variation related to X chromosome morphology in the "Moreton" race Obviously the differences between the "Moreton" and "Torresian" races are small relative to the variation within the races. This explains the impossibility of reliably recognising individuals of the two races in the field.

Because of the limited extent of the morphological differentiation between the races, the results are mainly of evolutionary rather than taxonomic significance, since the labour involved in a morphometric analysis is considerable and in this case the reliability of identification is not complete. The major interest lies in the fact that the karyotypic rearrangements which unambiguously distinguish the "Moreton" and "Torresian" races are associated with some morphological differentiation. This is a common finding in sibling species groups where a detailed morphological examination has revealed previously unnoticed differences between the species. Similarly morphological divergence has been detected in the Viatica group of the Morabine grasshoppers between taxa differing by as little as a single autosomal fusion. (Atchley and Cheney, 1974). These authors go so far as to state that their findings "refute the commonly held misconception among some cytologists that differentiation into chromosomal races is usually not accompanied by a corresponding amount of morphometric divergence". In the case of the races of *Caledia captiva*, the karyotypic divergence is very extensive and the morphological differentiation is much less so. Therefore it is difficult to state whether there is a correspondence between the two.

In the 16 group analysis of all four races, the "Daintree" race is clearly and significantly discriminated from the other three races, which cluster fairly closely together. Again the main axis of inter-racial differentiation is the third axis in the six variable analysis. Character 10, distance between the eyes at the vertex, has the heaviest weighting in the third variate. Characters 5 and 8 were eliminated from this analysis so the lack of separation between the "Moreton" and "Torresian" races is not surprising. There is some incipient resolution of the "south east Australian" race. This differentiation could be improved by separate analyses without extreme groups such as the "Daintree" race, since it is commonly found that the resolving power of canonical analysis is increased as extreme groups are eliminated (Dupraw, 1964; Thorpe, 1976). Unfortunately the interpopulation variation within the "Daintree" race has not been sampled, so it is possible that the discrimination of this population is fortuitous. However given the distance of this population from the others along the third canonical axis, this is unlikely.

The discovery of some inter-racial differentiation in both the 12 and 16 group analyses is fortunate because the alternative result of no variation is inconclusive and uninformative unless an exhaustive number and range of characters have been studied. If no inter-taxon variation is detected, it may simply mean that the particular characters chosen do not differentiate the taxa. Thus it is not valid to assume uniformity between sibling species until a large number of characters from a wide range of character systems are studied (Sokal, 1973).

In both the 12 and 16 group analyses, the second canonical variate means do not order the population centroids in a particularly meaningful way. In the 16 group analysis, the three "south east Australian" populations are separated from the rest in the second and third axes, but even the "Daintree" population is not distinct along the second axis. Character 4, width of the femoral stripe, has the greatest weight in this discriminant function vector in both cases. The percentage interpopulation variation accounted for by the second variate ranges from 15% to 24% in these two analyses. However in the 12 group analysis it makes no contribution at all to ordering the taxa and in the 16 group analysis, it makes only a minimal contribution Further at least in the 12 group analysis, it is not possible to relate this variate to other significant factors such as geographical distribution. Thus, although it is statistically significant, it has very little biological relevance. Similarly the fourth and subsequent axes in these analyses are not biologically meaningful.

The inclusion of *Caledia* species *nova 1* in the 17 group analysis has clearly demonstrated extensive differences between it and the races of *C. captiva*. Since it is discriminated from the rest along the first axis, which maximises interpopulation variation, the differences between the two taxonomically recognised species are obviously large relative to other interpopulation variation. The discriminant function vectors in the 17 group analysis are quite different from those in the previous two analyses and therefore the canonical variates cannot be compared. However the first canonical variate is at least partly related to size in the 17 group study, except that the relationship is the reverse of that observed in the 12 and 16 group analysis. The "Daintree" population is still discriminated from the other three races of *Caledia captiva* in the second and third variates. This emphasises the considerable difference between this race and the other three races, since the inclusion of an extreme group such as *C.* species *nova 1* would be expected to mask the more subtle inter-racial differences. The populations of the other three races cluster closely and are not clearly differentiated.

In summary, the qualitatively distinct *C.* species *nova 1* is clearly discriminated from all four races of *C. captiva* by the technique of canonical variate analysis using eight character variables. It does not show closer affinities to any one particular race and appears to be uniformly distinct from all four races. Of the four races of *Caledia captiva*, the "Daintree" race is the most distinct morphologically. It appears to have closest affinities with the "Torresian" race although it is difficult to judge the precise affinities. The "Torresian", "Moreton" and "South east Australian" races form a cluster with close affinities, although at least the "Torresian" and "Moreton" races are differentiated. It is not possible to assess the affinities within this group.

One of the principle advantages of canonical variate analysis is a reduction in the dimensionality of the population relationships. In these analyses, an attempt has been made to further reduce the dimensionality by removing those characters which contribute little to interpopulation discrimination in the first three variates. However characters should never be discarded simply in order to reduce the dimensionality of the relationships as this will result in an unnecessary loss of information and an inferior assessment of racial affinities.

(Thorpe, 1976). In only one case here has the exclusion of character variables led to an inferior discrimination between the races. In the analysis of the 12 group female data, the inter-racial discrimination along the third axis decreased when 5 characters were excluded. However there was a considerable improvement in inter-racial discrimination when the same five variables were excluded from the male data. In general, the *a posteriori* statistical criteria for the elimination of variables have led either to no decrease or a slight increase in the biologically meaningful resolution of taxa and hence are justified in these cases.

The absence of qualitative differences of the male genitalia between races is not surprising. F1 hybrids can be obtained easily in crosses, between the "Moreton" and "Torresian" races, so there are obviously no mechanical barriers to interbreeding between these races or indeed any of the races of *C. captiva*. The result is also in keeping with the general conservatism of the phallic complex in the subfamily Acridinae. However given the inter-racial morphometric differentiation in gross body characters, it is possible that there is some minor differentiation of the structures of the phallic complex which could only be detected by thorough morphometric analysis.

CHAPTER VII

GENERAL CONCLUSIONS

A major part of the study reported here has been concentrated on analysing those mechanisms which maintain the integrity of the 'Moreton" and "Torresian" races, in spite of hybridization and introgression of chromosomes from the "Torresian" into the "Moreton" race. The analysis of the isolating mechanisms has involved field studies of natural hybridization in the narrow contact zone between the races (Chapter III) and experimental hybridization in the laboratory (Chapter IV). Both the field and laboratory studies have revealed that fertile F1 hybrids (Chapter V) are produced in crosses between these races. However severe breakdown was observed in the laboratory reared F2 generation. In particular, the F2 progeny from reciprocal crosses between "Torresian" and "Moreton" metacentric X populations were found to be completely inviable. Furthermore, the statistical pattern of chromosomal associations in populations on the "Torresian" side of the contact zone, at least, (Chapter III) demonstrated deficiencies in the production of backcross progeny and provided evidence for severe backcross breakdown as well. Hybrid breakdown is therefore considered to be of crucial importance in maintaining the narrowness and stability of this hybrid zone and hence the integrity of the taxa.

Any explanation of hybrid breakdown must account both for the vigour, and in some cases, heterosis, displayed by the F1 hybrids and the severely reduced viability of the F2 and backcross progeny. If it is assumed that the two parental races or species have differently coadapted gene complexes, then the F1 hybrids will contain two different but complete haploid gene complexes or gametic complements. However, as a result of segregation and recombination, the individuals in the F2 and backcross generations will not, in general, possess complete coadapted complexes and as a consequence will suffer from reduced viability. Rare viable

F2 progeny will result when segregation, recombination and fertilization produce diploid complements similar to the parental or F1 hybrid complements. However abstract accounts of this sort are merely another means of describing the facts rather than explaining them (Maynard Smith, 1958). A more realistic and explanatory model of hybrid breakdown takes into account differences in linked developmental processes between the taxa. For example, if two developmental processes are related to each other, but have a different relationship in two closely related species, so that they are both fast in one species or both slow in the other, the F1 hybrids will survive if the genes controlling these processes are co-dominant or both the alleles of one species are dominant. The F1 hybrids will then be intermediate to the parents or resemble the parent with the dominant alleles, but in both cases will possess a developmentally compatible combination of genes. However only 37.5% of the F2 progeny will be viable on the co-dominant model and 62.5% on the dominant model. By increasing the number of developmentally linked loci, the severity of the breakdown can be increased.

Where hybrid breakdown is the major mechanism responsible for maintaining the integrity of two closely related taxa, the isolation will be at least potentially leaky if the breakdown is incomplete. The hybrid breakdown mechanism can then act as a selective semipermeable barrier, which will be the basis of a tension zone (Key, 1974). This selective barrier will allow some chromosomes or segments to pass from one species to another. Those chromosomes which do not possess genes incompatible with the genetic background of the recipient species or which have lost these genes by crossing over in the F1 hybrids, will occasionally segregate in such a way that they are not associated with other unfavourable combinations in the backcross generation. In other words, some novel segregants in the backcross generation or less likely the F2 generation, will be viable and capable of propagating this newly acquired variability. Moreover selective introgression of this sort will not lead to a gradual attrition of those barriers isolating the species, since the only chromosomes, which will be able to introgress, are those which do not cause unfavourable epistatic interactions in the backcross generation. These are, by definition, not involved in the hybrid breakdown mechanism. Thus the taxa involved can exploit the new variability derived by introgression, but, at the same time, they can also maintain isolation indefinitely and prevent the gradual fusion of the two gene pools.

The importance of introgressive hybridization in the evolution of animals is still contentious, despite the fact it is considered to be one of the commonest sources of new variability in actively evolving plant species (Stebbins, 1966). Mayr (1963) has stated a commonly held view that "the evolutionary importance of hybridization seems small in the better known groups of animals and reticulate evolution above the species level plays virtually no role in the higher animals". The greater developmental complexity of animals, which is controlled by integrated groups of coadapted genes, is considered to greatly reduce the probability of successful new combinations of genes and chromosomes arising after hybridizaition. However, even if the chance of successful introgression is small, it will nevertheless be of considerable biological and evolutionary significance if it introduces new genes into the recipient gene pool at a faster rate than mutation alone. Further, introgressive hybridization can also lead to the production of novel allelic variation that is not present in either of the parental species. Intragenic recombination in heterozygotes is theoretically capable of generating new alleles at rates several orders of magnitude above observed rates of standard mutational processes (Watt, 1972).

Although the evolutionary significance of introgression has been questioned, numerous examples clearly demonstrate that it does play a role, even in animals (Heiser, 1973). Indeed there are several clear examples of its occurrence among animals species. In the genus *Drosophila*, chromosomal analyses have demonstrated introgression between *D. americana* and *D. texana* (Patterson and Stone, 1962), *D. mojavensis* and *D. arizonensis* (Nagle and Mettler, 1969) and the sibling species *D. metzii* and *D. pellewae* (Pipkin, 1968, 1972). However, despite the many comprehensive studies over many years, it has never been detected in any natural populations of *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1973). In the Coleoptera, there is chromosomal evidence for introgression between *Pissodes approximatus* and *P. canadensis* (Manna and Smith, 1969) and also between *Chilocorus tricyclus* and *C. hexacyclus* (Smith, 1966). However despite hybridization in a 20-30 kilometre wide contact zone, introgression between *Chilocorus bipustulatus* and *C. geminus* is prevented because of hybrid breakdown (Zaslavski, 1963). Introgression has at least been demonstrated experimentally between the Dipteran species, *Dacus tryoni* and *D. neohumeralis* (Lewontin and Birch, 1966), although its importance in natural populations of these species has been questioned by Gibbs and Vogt (1977) (1969). Many cases of introgression of morphological characters are known from studies of bird species, for example between the red eyed towhees, *Piplio ocai* and *P. erythrophthalmus* (Sibley, 1954) and the grackles *Quiscalus quiscala quiscala* and *Q. q. versicolor* (Yang and Selander, 1968). In fact, 35 of the 517 extant species of North American birds are known to hybridize and backcross in contact zones (Short, 1972), and Short (1972) considers that hybridization has been a significant factor in the recent evolution of at least 15% of the Nearctic avifauna. Introgression may be of considerable importance in the evolution of fish, particularly freshwater species, where hybridization is very common (Hubbs, 1955). For example, there is both electrophoretic and chromosomal evidence showing introgressive hybridization between the Cyprinid species, *Gila orcutti* and *Hesperoleucus symmetricus* (Greenfield and Greenfield, 1972). Indeed, artificial introgressive hybridization has commonly been used in the *Xiphophorini* and the *Poeciliini* as a means of producing commercial strains of aquarium fish, and has often involved multiple backcrossing and sometimes even multiple species crosses (Kosswig, 1973).

As well as enhancing the genetic variation of the recipient species, introgression must also raise its fitness if the introgressed genetic material is to be maintained. In only two cases has the fitness of introgressed populations been measured relative to parental controls and in both cases it was possible to demonstrate a statistically significant enhancement of their fitness. Experimentally introgressed populations of *Drosophila mojavensis*, containing genetic material from *D. arizonensis*, are significantly fitter than monomorphic parental populations of either species in terms of productivity (Nagle and Mettler, 1969). Likewise laboratory populations of *Dacus tryoni*, containing introgressed material from *D. neohumeralis*, eventually displayed a superior fitness at higher temperatures after a one year period of equilibration of the introgressed genetic material (Lewontin and Birch, 1966). The latter experiment supports their hypothesis that *D. tryoni* had increased its range of ecological tolerance by introgression, even though the donor genes may not have been adaptive *per se*. In both of these cases, the introgressed genetic material was not initially coadapted with the recipient genome and must have been selected from a large number of possible new combinations, most of which would have been deleterious.

The apparent paradox of simultaneous isolation and introgression in the hybrid zone between the "Moreton" and "Torresian" races of *Caledia captiva* can be resolved by the model of asymmetrical hybrid breakdown (Chapter III). This model provides a satisfactory explanation of the results so far obtained, including the pattern of polymorphism near the contact zone in the "Moreton" race and the lack of polymorphism in the "Torresian" race (Chapter II), the narrowness of the hybrid zone and the pattern of non-random associations between non-homologous chromosomes in populations on the "Torresian" side of the zone. This is further supported by the experimental evidence presented in Chapters IV and V.

Importantly, the model generates testable predictions for further analysis, particularly concerning the behaviour of experimental backcrosses.

The superior survival of the "Moreton" backcross derivatives suggested by the hybrid zone data, would, on the model of asymmetrical breakdown, cause the hybrid zone to move and displace pure "Torresian" populations. As a consequence of the newly acquired "Torresian" genetic material, the "Moreton" populations should also increase their range of ecological tolerance, particularly to those conditions experienced only by the "Torresian" race. Thus the geographically restricted "Moreton" race would not face either ultimate genetic swamping or extinction by displacement (Key, 1974) by the "Torresian" race, but on the contrary would expand its distribution until an environmentally determined equilibrium position for the contact zone set the new limit for its distribution. The climatic gradient in south east Queensland (Chapter II) would provide an environmental gradient in which such an equilibrium could be established.

Although the hybrid zone between these races of *Caledia captiva* has been interpreted as a zone of secondary contact following genetic differentiation of the taxa in geographical isolation (Chapter II), alternative explanations have been proposed for related phenomena. In particular, several models of primary intergradation have been proposed. Here similar patterns of geographical distribution are considered to arise without the necessity of geographical isolation and the consequent absence of gene flow between the evolving taxa. Indeed, Endler (1977) considers that, in general, it is impossible to distinguish between primary and secondary intergradation, since, in his opinion, they will both produce the same patterns of geographical distribution and can evolve in a similar period of time.

The models of primary intergradation are of two related types. The stasipatric model of White (1968), which has already been discussed in some detail, postulates that a chromosomal rearrangement can arise within or at the periphery of the distributional range of a species, where it establishes itself. The derived form then expands its distribution and displaces the parental type by forming a narrow, moving hybrid zone. A necessary condition of this model is that the chromosomal rearrangements *per se* depress the fertility of the structural heterozygotes. However, it has been demonstrated that the 35 or more differences in centric position and pattern of C-banding, which exist between the "Moreton" and "Torresian" races, have no direct mechanical role in causing the observed meiotic anomalies in F1 hybrids (Chapter V). In any case, these meiotic anomalies will not seriously affect the ability of the hybrids to produce normal functional gametes, because of a gametic elimination system and particularly since some hybrids have similar levels of anomalies as the controls (Chapter V). For these reasons, the stasipatric model is clearly not applicable in this case. Furthermore, it must be pointed out that all similar cases, which have been presented as evidence for stasipatric speciation (White *et al.*, 1967; White *et al.*, 1969; Mrongovius, 1975; Craddock, 1971, 1975) have failed to provide the necessary evidence of significant hybrid infertility directly attributable to the chromosomal rearrangement differences between the taxa.

The alternative explanation of primary intergradation is the parapatric model of speciation (Bush, 1975; Endler, 1977). In this case, it is proposed that the species evolve as contiguous populations in a continuous cline, in which a narrow stepped cline or zone of hybridization then forms. Although there are interesting theoretical implications from this model, in which the steepening of the cline is attributed to modifier genes influencing the major genes, it is still unsatisfactory in several ways. First, the modifier genes are not defined in a biologically meaningful way. At least the stasipatric model proposes a feasible, if not generally applicable, explanation for the depression of fitness of heterozygotes as a result of meiotic disturbances due to chromosomal rearrangements. Secondly, given such modifiers do exist, the nature of their *de novo* occurrence is not discussed by Endler. It seems extremely unlikely that the appropriate modifiers would occur by chance in so many of the situations which Endler attempts to explain in this way. However, there are also specific objection to the applicability of this model to the hybrid zone between the "Moreton" and "Torresian" races of *Caledia captiva*. First, the multiple chromosomal differences between these races are not necessary and not expected on this model of speciation. Secondly, the presence of hybrid zones in *Caledia* and in several other groups of organisms in this area is better interpreted as the result of secondary contact following isolation in climatic refugia (Keast, 1962). The mesic wallum habitat of south east Queensland existed during the Pleistocene and would have provided a moist refuge area during the Pleistocene interpluvials (Coaldrake, 1961). Subsequent climatic amelioration would then permit the expansion of the fauna and flora trapped in this and other refugia and allow the establishment of secondary contact. The zones of contact between the species of "acid" frogs and the southern non-wallum species of the Genera *Litoria* and *Ranidella* have been interpreted in this way (Straughn and Main, 1966; Ingram and Corben, 1975). Endler (1977), however, considers that the presence of parallel clines in unrelated groups can still be explained by the parapatric model, if the organisms are responding to the same environmental gradients, but establish geographically different equilibrium positions for their narrow clines. However, even though the position of the hybrid zone in *Caledia* is established in a climatic gradient (Chapter II), the zones in the frog species mentioned previously have arisen because of different water pH requirements and tolerances of the taxa concerned. Thus the two hybrid zones, although parallel, have established their equilibrium positions in response to two totally different ecological conditions, namely seasonality of climate and water pH. These findings do, however, fit the climatic refuge hypothesis, since the requirement of acid water for breeding in the wallum frogs would evolve as a result of isolation in a mesic habitat, in which the nature of the soil caused a lowering of the water pH. This requirement would prevent expansion beyond the wallum of the frog fauna during wetter periods, whereas other groups without these requirements could expand their distribution.

Even on an allopatric model of speciation, one final problem remains. This involves the question of the origin and function of the large number of chromosomal differences which have been found between the obviously closely related "Torresian" and "Moreton" races of *Caledia*. However, on the hypothesis that chromosomal repatterning between taxa is based largely on divergent selection for "supergenes" or adaptive complexes of genetically linked loci (Darlington, 1940; Stebbins, 1950), these differences can be adequately explained as mechanisms for locking up adaptive complexes of genes. Furthermore, these functions of chromosomal rearrangements are consistent with the patterns of hybrid breakdown observed and also explain the nature of the isolating barriers which maintain the integrity of the "Moreton" and "Torresian" races of *Caledia captiva*.

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APPENDIX

Meiotic behaviour of a telecentric translocation heterozygote

1) Two chiasmata

a) two bivalents

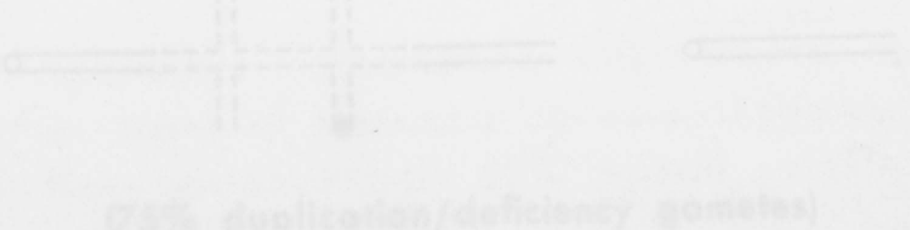
i)

(50% duplication/deficiency gametes)

ii)

(75% duplication/deficiency gametes)

b) one trivalent and one univalent



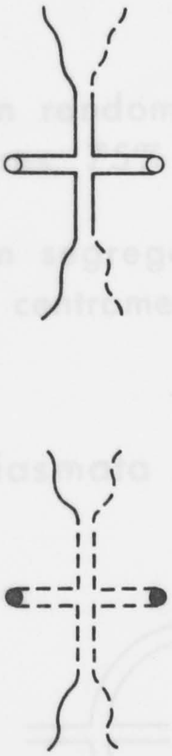
(75% duplication/deficiency gametes)

Meiotic behaviour of a telocentric translocation heterozygote

1) Two chiasmata

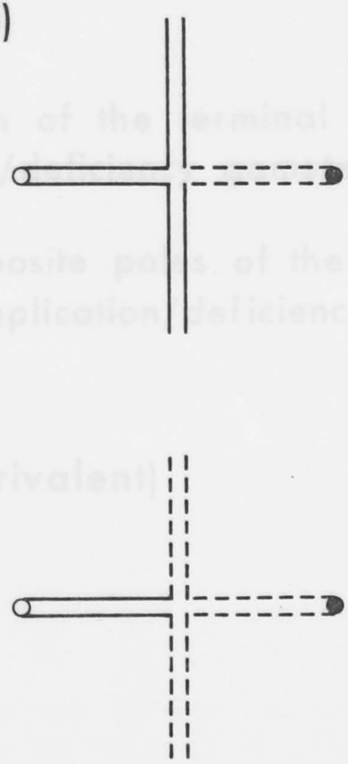
a) two bivalents

i)



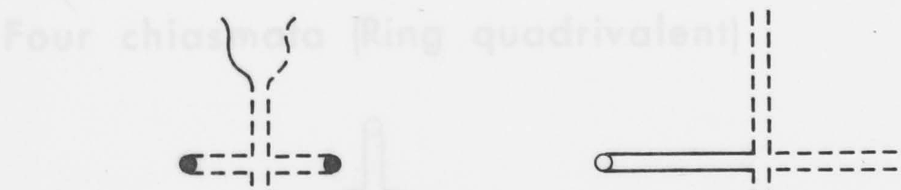
(50% duplication/deficiency gametes)

ii)



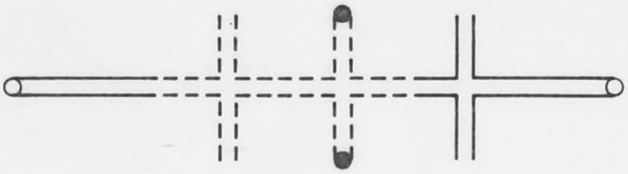
(75% duplication/deficiency gametes)

b) one trivalent and one univalent



(75% duplication/deficiency gametes)

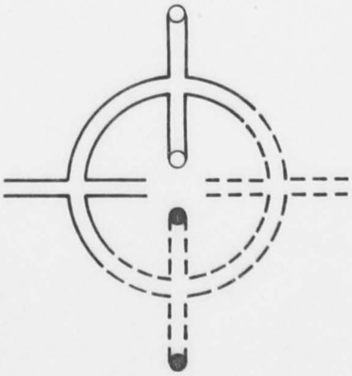
2) Three chiasmata (Linear quadrivalent)



i) (Given random segregation of the terminal centromeres, 75% duplication/deficiency gametes)

ii) (Given segregation to opposite poles of the terminal centromeres, 50% duplication/deficiency gametes)

3) Four chiasmata (Ring quadrivalent)



(50% duplication/deficiency gametes)

A telocentric translocation heterozygote, unlike other types of translocation heterozygotes, can, at best, produce only 50% normal gametes.